Journal of Trace Elements in Medicine and Biology 47 (2018) 98–103

Contents lists available at ScienceDirect

Journal of Trace Elements in Medicine and Biology

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

Evaluation of immunotropic activity of gold nanocolloid in chickens

Iwona Sembratowicz*, Katarzyna Ognik

Department of Biochemistry and Toxicology, Faculty of Biology, Animal Sciences and Bioeconomy, University of Life Sciences in Lublin, Akademicka 13, 20-950 Lublin, Poland

ARTICLE INFO

Keywords:
Nano-gold
Chickens
Blood
Immunity
Inflammation

ABSTRACT

Gold nanoparticles (AuNPs) are one of the most examined nanomaterials, but information about their immunogenic potential is still insufficient. Understanding interaction of AuNPs with immune system is essential in designing their safety and possibilities of biomedical applications. An experiment was conducted to determine immunotropic activity of gold nanocolloid (AuNPs) administered orally to chickens depending on dose and duration time. 162 birds were assigned to 9 experimental groups of 18 birds each. The control group (C) did not receive AuNPs. Groups: $T1_{0.5}$, $T1_{1.0}$, $T1_{1.5,}$ $T1_{2.0,}$ received nano-gold in a rate of 0.5 mg/kg body weight/d, 1.0 mg/kg body weight/d, 1.5 mg/kg body weight/d and 2.0 mg/kg body weight/d in 8–14, 22–28 and 36–42 days of the life. The birds in groups $T2_{0.5}$, $T2_{1.0}$, $T2_{1.5,}$ $T2_{2.0,}$ received nano-gold in the same doses, but only in 8–10, 22–24 and 36–38 days of life. Phagocytic activity of leukocytes was determined *in vitro* using *Staphylococcus aureus* 209P strain, their respiratory burst activity was quantified by nitroblue tetrazolium reduction test. Serum lysozyme content was determined by the turbidimetric method. The Wintrobe method was used to determine the erythrocyte sedimentation rate. Ceruloplasmin in the blood plasma was estimated by the p-phenylenediamine colorimetric method. The level of chicken immunoglobulins: IgA, IgM and IgY and interleukin IL-6 in the blood were determined using ELISA tests. The lowest dose of AuNPs, independently on duration time had no effect on immune parameters of chickens. In all other groups receiving nano-gold for a shorter period (T2), there was an increase in the respiratory burst activity of leukocytes and a drop in lysozyme activity in blood. The higher doses (1.5 and 2.0 mg/kg body weight/d) of the nano-gold administered for the longer time period had a pro-inflammatory effect, as indicated by an increase in the level of interleukin 6 and ceruloplasmin activity as well as the erythrocyte sedimentation rate. They also contributed to an elevation of class IgA and IgY contents in blood. The results of the study revealed that the influence of nano-gold on immune response of chickens were dependent both on dose and duration time. Long lasting administration of higher doses of AuNPs contributed to adverse effect in form of inflammation response. To avoid the development of inflammatory reaction, administered dose of nano-gold should not exceed 1.0 mg/kg body weight/d.

1. Introduction

Gold in bulk form is treated as inert, non-toxic and non-immunogenic, but it obtains new properties when its size is reduced to nanoscale (under 100 nm diameter). Metal nanoparticles possess the capacity to react with components of the immune system, suppress or stimulate it and also causing immunotoxic effects [1,2]. Gold nanoparticles (AuNPs), as yet has not been shown to have a cytotoxic effect on immunocompetent cells, but only rather an immunosuppressive effect [3–5]. In general, there is much conflicting information regarding nano-gold, on immune responses. A number of studies, indicate a stimulatory effect of AuNPs on the activity of macrophages, dendritic cells and peripheral blood phagocytes [6–8]. These cells easily absorb nanoparticles, which may lead to their stimulation and expression of pro-inflammatory cytokines, such as TNF-alpha, IL-1 and IL-6 [9]. Moreover, numerous authors indicate that nano-gold has an anti-inflammatory effect, manifested as a decrease in the level of cytokines and oxidative stress indicators [10–12]. In a study by Małaczewska [13], low, non-toxic doses of gold nanocolloid were shown to affect elements of specific immunity, as they stimulated proliferation of B lymphocytes isolated from the spleen of mice. An adjuvant effect of nano-gold manifested by enhancement of the response to vaccination was observed by Dykman et al. [14]. A study by Sengupta et al. [15] revealed immune-potentiating property of AuNPs which may be utilized in immune-deficient animals.

Despite the fact that many experiments have recently been conducted to evaluate the immunotropic properties of gold nanoparticles, knowledge concerning this question is still inadequate. It is also worth

* Corresponding author at: Department of Biochemistry and Toxicology, University of Life Science in Lublin, 13Akademicka Street, 20-950 Lublin, Poland.
E-mail address: iwona.sembratowicz@up.lublin.pl (I. Sembratowicz).

https://doi.org/10.1016/j.jtemb.2018.02.006
Received 21 November 2017; Received in revised form 26 January 2018; Accepted 5 February 2018

noting that these studies have primarily been conducted *in vitro* using established cell lines (mainly murine macrophages and human dendritic cells) or on rodents. There is a lack information about the influence of AuNPs on immunity of poultry. We assumed, that gold NPs absorbed into the blood may affect leukocytes, and depending of dose and time of exposure may modulate theirs activity and induce signal of inflammation. Therefore the aim of the study was to determine the effect of gold nanocolloid administered orally to chickens on activity of blood leukocytes and chosen inflammatory indices.

2. Materials and methods

2.1. Nanoparticles

The subject of the study was aqueous solutions of gold hydrocolloid at an initial concentration of 50 mg/l. The gold nanoparticles were non-ionic, nanocrystalline, chemically pure particles 5 nm in size, produced in a physical process (a non-explosive, high-current method for degradation of metals) by a unique patented technology licensed by Nano Technologies Group, Inc. (USA). All information about this product are included in European Patent Specification (EP 2 081 672 B1). Transmission electron microscope (TEM) images showed, that nanoparticles posses the shape of plates having a thickness of 3–5 atoms (Fig. 1), which significantly lowers their weight and ensures optimum active surface area (compared to spherical structures). On the basis of photographs taken by a transmission electron microscopes Tecnai G2 T20 X-TWIN (FEI, Hillsboro, USA) and LEO 912AB (Carl Zeiss GmbH, Jena, Germany), the average size of the gold nanoparticles was estimated at about 5 nm (Fig. 2).

2.2. Animals and experimental design

The material for the study consisted of one day-old Ross 308 chickens (♂) raised until their 42nd day of life. The experimental procedure was approved by the Second Local Ethics Committee for Experiments with Animals in Lublin (approval no. 30/2014). The birds were kept in pens on straw litter and reared in standard hygiene conditions in a building with regulated temperature and humidity. The chickens had permanent access to drinking water and received *ad libitum* complete feed mixes appropriate for the rearing period in accordance with feeding standards for poultry [16]. The experiment was carried out on 162 chickens assigned to nine experimental groups of 18 birds each (3 replications of 6 individuals each). The experimental design was presented in Table 1. The control group (C) did not receive gold nanoparticles. Birds in groups $T1_{0.5}$ i $T2_{0.5}$ received nano-gold at a rate 0.5 mg/kg body weight/d, in groups $T1_{1.0}$ and $T2_{1.0}$ at a rate

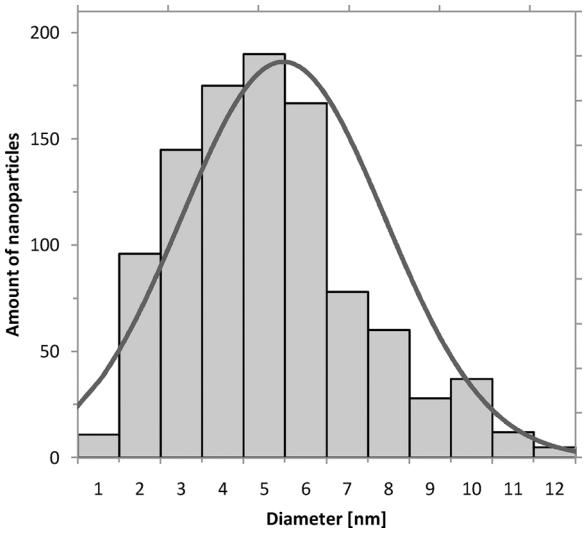

Fig. 2. Hydrocolloid of gold nanoparticles (Au-NPs) size distribution.

1.0 mg/kg body weight/d, in groups $T1_{1.5}$ and $T2_{1.5}$ at a rate1.5 mg/kg body weight/d and in group $T1_{2.0}$ and $T2_{2.0}$ at a rate 2.0 mg/kg body weight/d. Chickens in groups $T1_{0.5}$, $T1_{1.0}$, $T1_{1.5}$, $T1_{2.0}$, received gold nanoparticles via a tube into the crop in 3 cycles of seven days (8–14, 22–28 and 36–42 days of the life) and in groups $T2_{0.5}$, $T2_{1.0}$, $T2_{1.5}$, $T2_{2.0}$ in 3 cycles of three days (8–10, 22–24 and 36–38 days of life). We assumed that gold nanocolloid may act as immunostimulant, and continuous, long lasting administration may provide to adverse effects (overstimulation or dysregulation of immune system and even activation of autoimmune response). We also took into account the results of previous experiment, which revealed, that continuous application the highest rate (2.0 mg/kg body weight/d) of nano-gold had negative impact on hematological indices and growth parameters of chickens (not published data).

2.3. Laboratory analysis

At the end of the study the samples of blood were collected for analysis from the wing vein of 12 chicks from each group (4 birds x 3 replications). The immunological analyses involved determination of the phagocytic activity of leukocytes against the *Staphylococcus aureus* 209P strain, expressed as the percentage of phagocytic cells (% PC) and the phagocytic index (PI) [17]. The respiratory burst activity of heterophils and monocytes was quantified by nitroblue tetrazolium reduction (NBT) to formazan as a measurement of production of oxygen

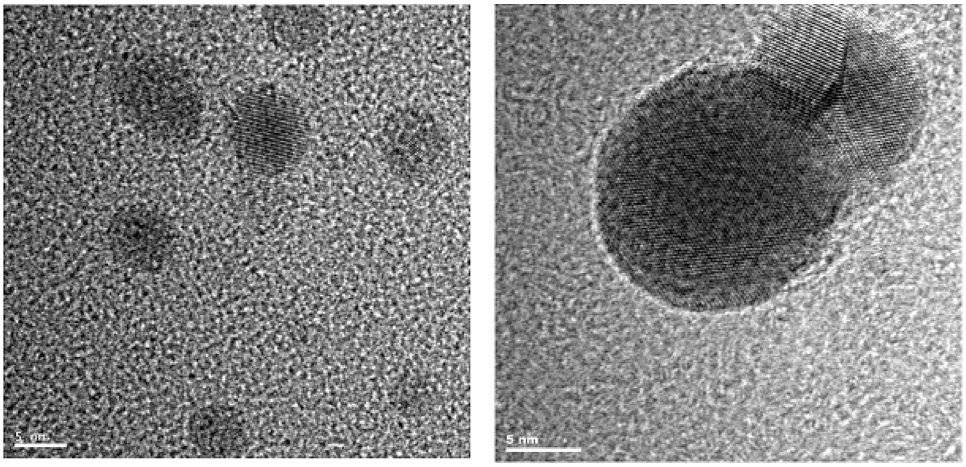

Fig. 1. Transmission electron microscopy (TEM) images of gold nanoparticles (Nano Technologies Group, Inc.).