IRON NANOPARTICLES’ EFFECTIVENESS FOR NEW ANTIANEMIC PREPARATIONS DEVELOPMENT

Liudmyla RIEZNICHENKO1, Andrii DOROSHENKO2, Tamara GRUZINA1, Svitlana DYBKOVÀ1, Zhanna POLOVA2, Zoya ULBERG1, Ivan CHEKMAN1,2

1F.D. Ovcharenko Institute of Biocolloidal Chemistry of NAS of Ukraine, Kyiv, Ukraine; Reznichenko_LS@mail.ru
2O.O. Bohomolets National Medical University, Kyiv, Ukraine; amdor@mail.ru

Abstract

In spite of considerable quantity of antianemic preparations with different iron forms and additives, which are presented on modern pharmaceutical market, iron deficiency anemia is one of the most wide-spread pathological states as well as social problems in the world. Pregnant women, children and elderly persons are particularly vulnerable. The situation is complicated by the presence of some disadvantages and drug side effects of existing antianemic medicines. So, development of new antianemic preparations is very perspective. Iron nanoparticles are possessed by high potential in this area.

For the purpose of new effective antianemic preparations’ creation the technology of colloidal-chemical synthesis in water medium for 40 nm spherical iron nanoparticles has been developed. Synthesized iron nanoparticles have been characterized as biosafe using in vitro and in vivo tests. For biosafety level estimation parameters of cytotoxicity, genotoxicity, mutagenicity and main biochemical markers have been used. LD50 of synthesized nanoparticles in per os dosing (BALB/c mouse line) is more than 5 g/kg.

Biological activity of the iron nanoparticles as potential pharmacological substance with antianemic properties has been studied on the model of iron deficiency anemia with Wistar rats and BALB/c mice females using. It has been shown reliable increasing of main blood parameters such as hemoglobin level, iron concentration in blood serum, transferrin saturation percentage up to normal level of healthy animals comparatively with control within 10 days of experimental treatment course under the conditions of peroral as well as intravenous route of iron nanoparticles’ introduction.

Keywords: Iron nanoparticles, biosafety, antianemic preparation, effectiveness.

1. INTRODUCTION

Iron deficiency anemia (IDA) is one of the most wide-spread pathological states as well as social problems in the world according to the World Health Organization (WHO) data. The deficiency of iron is just one nutrient deficiency which is significantly prevalent not only in developing countries, but also in industrialized ones. This pathological condition is particularly significant for the most vulnerable groups of population among which women of reproductive age, and especially pregnant women, children and elderly persons. The WHO statistics indicates the enormous numbers: 2 billion people – over 30% of the world’s population – are anemic [1, 2].

Because of the necessity of iron deficiency control modern pharmaceutical market presents considerable series of antianemic preparations, which differ in iron form and/or concentration characteristics as well as in presence and allowance of the additives. Unfortunately, in most cases all types of these preparations are possessed by some disadvantages and drug side effects among which low bioavailability, nausea, anorexia, metal taste in mouth, constipation, protracted course of therapy (2–3 months) for achievement of therapeutic efficiency, etc. [2–4].
So, search and development of new class pharmacological substances with antianemic properties for effective struggle with IDA is very urgent.

Iron nanoparticles are possessed by high potential in this area according to the well-known biological activity of metal nanoparticles on the molecular level.

The synthesis of biosafe and biocompatible iron nanoparticles and their study as potential antianemic pharmacological substance for new class antianemic preparations’ development was the main goal of this work.

2. MATERIALS AND METHODS

The technology of colloidal-chemical synthesis in water medium for iron nanoparticles (FeNP) obtaining has been developed in F.D. Ovcharenko Institute of Biocolloidal Chemistry. The shape and size of synthesized FeNP has been defined by the method of transmission electron microscopy (TEM). FeNP biosafety and biocompatibility parameters with using wide spectrum in vitro and in vivo criteria of cytotoxicity, genotoxicity, mutagenicity, main biochemical markers have been studied for completing of the passport of nanomaterial.

All experiments with model animals have been carried out in compliance with “Guide for the Care and Use of Laboratory Animals”. Experimental animals have been housed on the standard regime.

Oral acute toxicity (LD_{50}) of iron nanoparticles has been assessed on female BALB/c mice during 14 days according to the limit test procedure at 5000 mg per kg dosing (OECD guidelines).

Biological activity of the iron nanoparticles as potential pharmacological substance with antianemic properties has been studied on the model of iron deficiency anemia using Wistar rats’ females.

Iron deficiency anemia in experimental animals was modeled using iron deficiency diet. Control intact animals received a diet with normal iron content. Animals of all groups were watered with distilled water.

The experimental treatment courses of the model IDA treatment included the conditions of peroral and intravenous routes of iron nanoparticles’ introduction.

The experimental animals have been divided into 7 groups:

- Control group of healthy animals without treatment;
- Control group of the anemic animals with model IDA without treatment;
- Experimental group of the anemic animals treated with therapeutic dose (12.0 mg/kg per day) of peroral iron nanoparticles’ introduction during 10 days;
- Experimental group of the anemic animals treated with the dose in 1/10 of therapeutic dose (1.2 mg/kg per day) of peroral iron nanoparticles’ introduction during 10 days;
- Experimental group of the anemic animals treated with therapeutic dose (12.0 mg/kg per day) of peroral comparison drug introduction during 10 days;
- Experimental group of the anemic animals treated with 5 intravenous injections of iron nanoparticles in the dose 12.0 mg/kg per day in terms of 1 injection within 3 days;
- Experimental group of the anemic animals treated with 5 intravenous injections of iron nanoparticles in the dose 1.2 mg/kg per day in terms of 1 injection within 3 days.

The level of main blood parameters has been monitored:

- after 1\textsuperscript{st}, 5\textsuperscript{th}, and 10\textsuperscript{th} days of the substance peroral introduction;
- after 1\textsuperscript{st}, 3\textsuperscript{rd}, and 5\textsuperscript{th} intravenous injections of the substance.

The therapeutic dose for experimental anemic animals has been calculated on the basis of daily therapeutic iron dose for human (200 mg), which is usually recommended in IDA treatment, with a glance of species’ resistibility factor.

As comparison drugs some commercial preparations based on pharmacological substance – ferri (III) hydroxydi polymaltosum complexus – have been used.
The rats were euthanized by decapitation under anesthesia.

Blood parameters - hemoglobin level, iron concentration in blood serum, transferrin saturation percentage - have been analyzed using Filicit-Diagnostics kits (Ukraine).

The status of microflora in lower part of model animals’ gastrointestinal tract after 10 days course of FeNP introduction, in compare with comparison drug, has been determined using standard microbiological protocols.

3. RESULTS AND DISCUSSION

Water dispersion of FeNP has been synthesized with the aim of its potential antianemic properties’ estimation.

FeNP with spherical shape and average size 40 nm have been synthesized.

According to the experimental data, obtained using wide spectrum of in vitro and in vivo biosafety tests, the synthesized FeNP have been characterized as biosafe and biocompatible. They were noncytotoxic, nongenotoxic, nonmutagenic and biosafe according to the main biochemical markers. The TEM images of obtained FeNP are presented on Figure 1.

![Transmission electron microscopy (TEM) image of synthesized 40 nm spherical iron nanoparticles](image)

**Fig. 1** Transmission electron microscopy (TEM) image of synthesized 40 nm spherical iron nanoparticles

LD$_{50}$ of synthesized 40 nm iron nanoparticles in per os conditions (BALB/c mouse line) exceeds 5000 mg/kg value. None of animals died during 14 days of post exposure period, as well as no signs of toxicity have been found under the limit test procedure.

FeNP antianemic properties have been studied on the alimentary model of iron deficiency anemia.

It has been shown reliable increasing of main blood parameters such as hemoglobin level, blood serum iron concentration, and transferrin saturation percentage up to normal level of healthy animals comparatively with anemic control within experimental treatment course under the conditions of peroral as well as intravenous route of iron nanoparticles’ introduction.
The effectiveness of comparison drugs based on pharmacological substance ferri (III) hydroxydi polymaltosum complexus was reliably lower according to the analyzed blood parameters.

The absence of dysbacteriosis and constipation (widespread side effects for existing commercial antianemic iron preparations) has been observed under the treatment course of experimental FeNP introduction.

It has been revealed, that according to the estimated blood parameters the synthesized 40 nm FeNP are possessed by expressed antianemic activity in the dose that is 10 times lower than the generally accepted therapeutic one.

4. CONCLUSION

The synthesized 40 nm spherical FeNP are possessed by expressed antianemic properties as well as some benefits comparatively with the drugs of comparison, among which:

- high biological safety and low toxicity according to in vitro and in vivo tests;
- remarkable antianemic activity in the dose that is 10 times lower than the generally accepted therapeutic dose;
- favourable effects on the gastrointestinal tract (the absence of side effects such as constipation and intestinal dysbacteriosis).

Such properties and benefits indicate significant potential of the synthesized FeNP as pharmacological substance with antianemic properties for new class antianemic preparations’ development.

At the same time the explanation of molecular mechanisms of the iron nanoparticles biological activity is necessary condition for their effective medical application.

LITERATURE