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Assessment of the effect of iron-rich humic substances on hematological parameters in the model of acute posthemorrhagic and iron deficiency anemia

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ABSTRACT

Aim. To assess the effect of iron-rich humic substances on hematological parameters in acute post-hemorrhagic and iron deficiency anemia.

Materials and methods. Materials for the study were samples of iron-rich active pharmaceutical ingredients based on humic substances (Fe(III) hydroxide complexes with humic substances and polymaltose): HA-Fe^{3+}, HA-PM-Fe^{3+}, FA-Fe^{3+}, and FA-PM-Fe^{3+}. The anti-anemic activity of the substances was studied on 53 female Wistar rats of the conventional rat line in the model of acute posthemorrhagic and iron deficiency anemia. Anti-anemic activity was assessed by the hemoglobin level, erythrocyte count, hematocrit, and serum iron level.

Results. The studied substances HA-Fe^{3+} and FA-Fe^{3+} are the most effective in correcting the consequences of both experimental acute posthemorrhagic anemia and iron deficiency anemia. Their effect is comparable to that of the positive control drug Ferrum Lek.

Conclusion. Fe(III) hydroxide complexes stabilized by humic and fulvic acids exhibit anti-anemic activity.

Keywords: acute posthemorrhagic anemia, iron deficiency anemia, humic substances, ligands, Fe(III) hydroxide complexes

Conflict of interest. The authors declare the absence of obvious and potential conflicts of interest related to the publication of this article.

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Оценка влияния железосодержащих субстанций на основе гуминовых веществ на гематологические показатели на модели острой постгеморрагической и алиментарной анемии

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РЕЗЮМЕ

Цель. Оценить степень влияния железосодержащих субстанций на основе гуминовых веществ на гематологические показатели при острой постгеморрагической и алиментарной анемии.

Материалы и методы. Образцы железосодержащих активных фармацевтических субстанций на основе гуминовых веществ (комплексы гидроксида Fe(III) с гуминовыми веществами и полимальтозатом): ГК-Fe^{3+}, ГК-ПМ-Fe^{3+}, ФК-Fe^{3+} и ФК-ПМ-Fe^{3+}. Противоанемическая активность субстанций исследована на 53 самках крыс линии Вистар конвенциональной категории на модели острой постгеморрагической и алиментарной анемии. Противоанемическая активность оценена по показателям: уровень гемоглобина, содержание эритроцитов, гематокрит и уровень сывороточного железа.

Результаты. Исследуемые вещества ГК-Fe^{3+} и ФК-Fe^{3+} являются наиболее эффективными в коррекции последствий как экспериментальной острой постгеморрагической анемии, так и алиментарной анемии. Их эффект сопоставим с препаратом положительного контроля «Феррум Лек».

Заключение. Комплексы гидроксида Fe(III), стабилизированные гуминовыми кислотами и фульвокислотами, проявляют антианемическую активность.

Ключевые слова: острая постгеморрагическая анемия, железодефицитная анемия, гуминовые вещества, лиганды, комплексы гидроксида Fe(III)

Конфликт интересов. Авторы декларируют отсутствие явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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Соответствие принципам этики. Эксперименты на животных соответствуют этическим нормам и принципам биомедицинских исследований и одобрены этическим комитетом СибГМУ (протокол № 8461/1 от 05.11.2020).

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INTRODUCTION

High biological value of iron and significance of this transition metal and its compounds are proven by high abundance of iron in nature in general and in living beings in particular (as it is a cofactor of more than 70 metalloenzymes and porphyrins), electron-donating and electron-withdrawing properties of its ions, and its participation in complex metabolic pathways (hematopoiesis, glycolysis, cellular respiration, detoxification, etc.) [1–3]. Functional iron deficiency (mainly, in the composition of hemoglobin) and decreased iron in macrophages and hepatocytes contribute to the emergence of iron deficiency anemia (IDA), which is diagnosed in almost every tenth person in the world, according to WHO [4–7].

The clinical significance of IDA is associated not only with the pandemic prevalence, but also with the adverse effect of IDA on the most vulnerable to this disease age groups, including children, women of reproductive age, pregnant women, and the elderly [3–15]. They have a history of growth retardation, hydrops, premature and complicated births, inflammatory processes, decreased physical activity and disability, cognitive impairment, mental impairment, etc. [6, 7, 10].

Long-term oral iron therapy is one of the fundamental principles of sideropenia therapy, set forth by L.I. Idelson back in the 1980s [3] and described in the Russian "Clinical guidelines for Iron Deficiency Anemia Management 2020" [3]. Moreover, patients should not discontinue to take drugs when the hemoglobin level and the erythrocyte count are restored. Instead, they should continue taking medications in the adjusted dose until the iron level is restored, which should last for at least 6 months.

Currently, the pharmaceutical market has more than 30 monocomponent and combined drugs of ferrous (mainly sulfate) and trivalent iron (hydroxide polymaltose complex) and about 70 multivitamin drugs [3]. Due to various chemical structure, iron preparations can differ significantly in terms of bioavailability. Absorption of iron(II) (in the form of sulfate and fumarate) occurs along a concentration gradient in the intestinal region (passive diffusion) and exceeds the rate of adsorption of iron(III), which ultimately can increase the level of serum Fe^{2+} and cause intensification of oxidative processes (oxidative stress in the gastrointestinal tract is observed in more than 20 % of patients) [3, 6, 8, 10].

Moreover, adverse drug reactions take place, such as erythema, a metallic taste in the mouth (as severe as ulceration of the mucous membrane), darkening of tooth enamel and gums, dyspepsia (nausea, vomiting, diarrhea, constipation, etc.), and epigastric pain [3, 10]. Low digestibility of trivalent iron ions is associated with their ability to hydrolyze in the gastrointestinal tract with the formation of a precipitate. The iron(III)-hydroxide polymaltose complex (the active ingredient of such drugs as Ferrumlek, Maltofer, Ferinject) is a promising molecule among the compounds of iron(III), as it overcomes the enterocyte membrane barrier by means of active transport, therefore, the risk of siderosis development is reduced [6]. Moreover, it is redox – inert and does not interact with food components and other drugs [4]. Its only drawback is that patients may develop allergic reactions, which can be as severe as anaphylactic shock [3, 8]. Therefore, there is an urgent need to search for new promising ligands for iron(III) ions used for prevention and therapy in manifest iron deficiency, which include humic substances (HS) [16–23].

HS are refractory polydisperse copolymers, which are carriers of a large number of functional groups, including carboxyl, phenolic, quinoid, amide, ester, ether, etc. [16]. Polyfunctionality, developed inner surface, and, consequently, high reactivity (the ability to participate in ionic, redox reactions, van der Waals interactions, etc.) determined the affinity of HS to cells of various organs and systems, and their natural origin ensured safety, the absence of toxic effects in large concentrations, and a mild effect on metabolic processes at the cellular, organ, and organ system levels. Due to all this, HS are used as hepatoprotectors, anti-inflammatory and immunomodulatory agents [17], antihypoxants, antioxidants [18, 19], and detoxification and anti-allergic agents [20].

This class of natural biomolecules is now actively studied in a wide range of research areas because of the ability of HS to act as polydentate ligands due to electron-donating groups and slow down the migration of toxic metals in natural biological media. At the same time, a number of studies established a strong correlation between metal toxicity and the capacity of HS to bind metal ions [21, 22]. HS can act not only as effective chelate sorbents [23], but also as carriers of biogenic metals (namely, iron) in an easily accessible complex form, which, along with the absence of enzymatic systems in the body that metabolize them, contributes to a longer therapeutic effect.

In this regard, the aim of this study was to assess the effect of iron-containing active pharmaceutical ingredients (API) based on complexes of iron(III) hydroxide with HS on hematological parameters in the model of acute posthemorrhagic and iron deficiency anemia.

MATERIALS AND METHODS

In the experiment, samples of iron-containing API based on HS (complexes of iron(III) hydroxide with HS and polymaltose) were synthesized in the Laboratory of Natural Humic Systems of the Department of Medical Chemistry and Fine Organic Synthesis, Faculty of Chemistry, Lomonosov Moscow State University: HA-Fe^{3+} (with potassium humate), HA-PM-Fe^{3+} (with potassium humate and polymaltose (1 : 1)), FA-Fe^{3+} (with fulvic acid), and FA-PM-Fe^{3+} (with fulvic acid and polymaltose (1 : 1)). The first stage of the study involved screening of the anti-anemic properties of the substances in order to find the most effective candidate drugs to treat acute posthemorrhagic anemia (APHA) and was performed on 30 female Wistar rats of the conventional rat line (weighing 270–310 g). The animals were kept, cared for, and treated in accordance with the recommendations of international ethics committees. The protocol of experiments carried out in this study complied with the ethical standards and principles of biomedical research and was approved by the Ethics Committee at Siberian State Medical University (Protocol No. 8461/1 of 05.11.2020).

At the first stage, the animals were randomly divided into 6 experimental groups; in each group, APHA was caused by blood loss (the sample volume of the collected biofluid was 1.5 % of body weight) [24]. Interspecies dose conversion was performed to calculate the dose of iron to be administered [25]. The animals of group 1 (control, $n = 5$) were intragastrically injected with 1 ± 0.1 ml of purified water for 5 days after acute blood loss. The animals of group 2 were intragastrically injected with the reference listed drug Ferrum Lek® syrup (Slovenia) at a dose of 17 mg / kg of elemental iron ($n = 5$ positive control, control-3). The animals of groups 3, 4, 5, and 6 ($n = 5$) were intragastrically injected with iron-containing APIs based on HS (HA-Fe^{3+}, HA-PM-Fe^{3+}, FA-Fe^{3+}, FA-PM-Fe^{3+}, respectively) at a dose of 17 mg / kg of elemental iron for 5 days. Blood from the caudal vein of the animals of all groups was taken on day 6; euthanasia by CO_2 asphyxiation then followed. Next, hematological tests of the collected biological material were performed to assess hemoglobin level (HGB), g / l; erythrocyte count (RBC), $*10^3$ / μl, and hematocrit (HCT), %. Spectrophotometry was used to determine the content of serum iron (reagents "Vector-Best" (Novosibirsk), spectrophotometer SF-2000 (Russia)).

At the second stage, we studied the effect of the most effective preparations based on HS, identified during the first stage of the experiment, on the course of IDA. A total of 23 female Wistar rats of the conventional rat line weighing 216–256 g were used in the experiment. During 4.5 months, part of the animals (5 animals) had unlimited access to complete feed with a normal iron content (76.8 μg / g), and 18 female rats received *ad libitum* diet with a low iron content (28.2 μg / g). After 4.5 months, the main hematological parameters were measured in all animals (see above). After that, five animals were intragastrically injected with 1 ± 0.1 ml of purified water for 14 days. The other 18 animals with developed anemia were divided into three groups ($n = 6$), and in the next 2 weeks of the experiment, they continued to receive a low-iron diet while getting daily intragastric injections of the reference listed drug Ferrum Lek® syrup (Slovenia), HA-Fe^{3+}, and FA-Fe^{3+}, respectively. The iron content in Ferrum Lek®, HA-Fe^{3+}, and FA-Fe^{3+} was 17 mg / kg of elemental iron. After that, blood was taken from the caudal vein (followed by CO_2 asphyxiation), and morphological and biochemical parameters were measured (see above).

Statistica 8.0 (StatSoft Inc.) was used for the statistical data analysis. Methods of non-parametric statistics were used in the study: namely, the Friedman and Kruskal – Wallis tests. For each sample, we calculated the mean value X and the error of the mean SE. Differences between observations / groups were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

Following the data analysis, it was found that acute blood loss in rats of the control group led to a drop in the studied hematological parameters. At the same time, these parameters did not reach the corresponding baseline values when purified water was used further on in the experiment. In the group of the positive control (after APHA, the reference listed drug Ferrum Lek® was used), a significant increase in hemoglobin, hematocrit, and RBC levels was observed compared with animals in the control group, but the parameters also did not reach the baseline level ($p < 0.05$) (Table 1).

Table 1

Hematological parameters after 5 days of the experiment, $X \pm SE$

Group	Before a course of medication (control-1)				After a course of medication			
	HGB, g / l	HCT, %	RBC, $\times 10^3$ / mcl	Fe, µmol / l	HGB, g / l	HCT, %	RBC, $\times 10^3$ / mcl	Fe, µmol / l
Water, $n = 5$ (control-2)	176.9 ± 2.0	45.1 ± 0.8	8.1 ± 0.1	61.7 ± 2.2	149.4 ± 2.1*	43.2 ± 0.5*	7.4 ± 0.1*	47.3 ± 1.7*
Ferrum Lek®, $n = 5$ (control-3)	189.4 ± 5.3	46.6 ± 1.8	8.5 ± 0.4	64.0 ± 8.9	158.4 ± 6.9*	45.8 ± 2.1	7.6 ± 0.3	44.4 ± 2.9
HA-Fe^{3+}, $n = 5$	178.8 ± 3.9	47.0 ± 2.3	8.4 ± 0.4	74.4 ± 6.4	158.4 ± 7.1*	45.8 ± 2.1	7.6 ± 0.4	51.6 ± 5.8*
HA-PM-Fe^{3+}, $n = 5$	191.1 ± 13.3	43.6 ± 1.6	7.8 ± 0.3	56.3 ± 7.0	146.0 ± 2.9*	42.6 ± 0.8	7.1 ± 0.3	38.8 ± 7.3
FA-Fe^{3+}, $n = 5$	182.1 ± 6.1	43.9 ± 3.5	7.9 ± 0.6	60.6 ± 2.2	145.3 ± 8.4*	42.6 ± 2.3	7.2 ± 0.6	48.0 ± 7.1
FA-PM-Fe^{3+}, $n = 5$	172.1 ± 3.0	43.5 ± 2.8	7.9 ± 0.4	58.7 ± 3.9	143.6 ± 6.8*	41.4 ± 1.9	7.1 ± 0.4	51.1 ± 4.4

The differences were statistically significant, $p < 0.05$: * with control-1 (the Friedman test); # with control-2 (the Kruskal – Wallis test); ^ with control-3 (the Kruskal – Wallis test).

Some hematological parameters in the rats were normalized after intragastric administration of the studied API at a dose of 17 mg / kg / day for 5 days after modeling APHA. It should be noted that during intragastric course administration, the studied substances HA-Fe^{3+} and FA-Fe^{3+} demonstrated greater efficiency in correcting the consequences of experimental APHA in comparison with samples of iron-containing APIs HA-PM-Fe^{3+} and FA-PM-Fe^{3+}, as the former resulted in a more significant increase in the levels of hematological parameters under study than the latter. At the same time, all the studied samples of iron-containing APIs based on HS had the same effect as the positive control drug Ferrum Lek®, which led to effective normalization of hematological parameters in the laboratory animals. No significant differences were found between the efficiency of the studied substances and the reference listed drug.

Following daily intragastric administration of HA-Fe^{3+} and FA-Fe^{3+}, as well as the reference listed drug Ferrum Lek® for 14 days at a dose of 17 mg / kg of elemental iron, serum iron concentrations returned to the baseline values ($p < 0.05$) (Table 2). It should be noted that the course administration of HA-Fe^{3+}increased the serum iron concentration in comparison with Ferrum Lek®, which was observed as a consistent trend ($p > 0.05$) (Table 2). However, administration of the studied substances and the reference listed drug for 14 days did not allow to bring hemoglobin and hematocrit levels to the control values ($p < 0.05$) (Table 2). It should also be noted that course administration of Ferrum Lek®, HA-Fe^{3+}, and FA-Fe^{3+} at a dose of 17 mg / kg / day for 14 days did not affect the body weight of rats and the absolute and relative weights of the liver and spleen in the model of IDA ($p > 0.05$).

Table 2

Hematological parameters after 14 days of the experiment, $X \pm SE$

Group	After modeling anemia				After a course of medication			
	HGB, g / l	HCT, %	RBC,$\times 10^6$/ mcl	Fe, µmol	HGB, g / l	HCT, %	RBC,$\times 10^6$ / mcl	Fe, µmol
Water, $n = 5$	198.8 ± 3.2	59.6 ± 0.9	10.3 ± 0.2	64.5 ± 1.6	197.8 ± 4.5^	59.5 ± 1.7^	9.7 ± 0.3	67.3 ± 1.1
Ferrum Lek®, $n = 6$	170.6 ± 6.7	51.1 ± 2.2	10.0 ± 0.4	25.4 ± 5.8	169.6 ± 3.2#	50.3 ± 0.9#	9.5 ± 0.3	60.6 ± 8.0*
HA-Fe^{3+}, $n = 6$	180.8 ± 5.7	53.6 ± 1.7	10.0 ± 0.4	34.1 ± 3.7	172.0 ± 1.9#	51.3 ± 0.6#	9.4 ± 0.1	75.0 ± 6.7*
FA-Fe^{3+}, $n = 6$	164.8 ± 11.7	50.1 ± 3.3	10.2 ± 0.5	20.4 ± 5.5	166.0 ± 1.7#	49.7 ± 0.7#	9.3 ± 0.2	62.3 ± 7.9*

The differences are statistically significant, $p < 0.05$: * with the group "After modeling anemia" (the Friedman test); # with the group "Water" (the Kruskal –Wallis test); ^ with the group "Ferrum Lek" (the Kruskal – Wallis test).

CONCLUSION

It was found that daily intragastric administration of the studied APIs after modeling APHA contributed to partial normalization of hematological parameters. Among the four samples studied, the most pronounced increase in hemoglobin concentration, erythrocytosis, and serum iron was observed in two iron-containing samples of APIs, namely, HA-Fe^{3+} and FA-Fe^{3+}. At the same time, the effect of all samples of iron-containing APIs based on HS was similar to the positive control drug Ferrum Lek®. No significant differences were found between the efficiency of the studied substances, as well as the reference listed drug.

The anti-anemic activity of iron(III) hydroxide complexes stabilized by humic and fulvic acids was

shown on the model of chronic IDA. At the same time, both APIs (HA-Fe^{3+}, whose matrix is 100% potassium humate, and FA-Fe^{3+}, whose matrix is 100 % fulvic acids) exhibit comparable activity. The results obtained confirmed the prospects of using HS as ligands in order to obtain APIs to normalize IDA.

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Bratishko K.A., Ufandeew A.A., Buyko E.E., Rabcevich E.S., Kuznecova M.V. – carrying out of the experiment, analysis and interpretation of the data. Logvinova L.A. – analysis and interpretation of the data, drafting of the manuscript. Ivanov V.V. – formulation of the aim and objectives, carrying out of the experiment, analysis and interpretation of the data. Zhirkova A.M. – synthesis and modification of the studied compounds. Zima A.P., Belousov M.V. – formulation of the aim and objectives, analysis and interpretation of the data. Perminova I.V. – conception and design, synthesis and modification of the studied compounds. Zykova M.V. – conception and design, formulation of the aim and objectives, critical revision of the manuscript for important intellectual content, editing of the manuscript, presentation of the published work.

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