Journal of Biomaterials and Nanobiotechnology, **2011, 2, 557-560**
doi:10.4236/jbnb.2011.225066 Published Online December 2011 (http://www.scirp.org/journal/jbnb)

Study of Safety of Molybdenum and Iron-Molybdenum Nanoclaster Polyoxometalates Intended for Targeted Delivery of Drugs*

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Received September 16th, 2011; revised October 29th, 2011; accepted November 20th, 2011.

ABSTRACT

Nanocluster polyoxometalates with a structure of buckyball (*fullerene*) *are a promising means of targeted delivery of drugs in the body. In this paper*, *based on an analysis of histological sections of liver and kidney and peripheral blood*, *showed a significant reduction of toxicity of buckyballs*, *containing ions of molybdenum* (*VI*), *the substitution of Mo* (*V*) *ions to Fe* (*III*), *in contrast to buckyballs based on Mo* (*V*). *The absence of accumulation of molybdenum in rats with a daily intramuscular injection of aqueous solutions of both drugs within a month was confirmed.*

***Keywords*:** *Buckyball*, *Polyoxometalates*, *Direct Delivery*, *Toxicity*

1. Introduction

The search for effective and safe means of targeted delivery of drugs is one of the most pressing issues of nanobiotechnological studies [1-3]. Candidates for the role nanocontainers or nanokernel is a group of compounds, united by the name of polyoxometalates (POM) with the structure of keplerates (buckyballs or fullerenes) [4,5]. There is a number of prerequisites for success when using this type of compounds: water solubility, an ability to reversibly absorb various organic compounds [6], to form complexes [7], an ability to decompose within a certain time [6,7] and move under the influence of weak electric fields [6].

One of the most typical buckyball representatives is molybdenum buckyball Mo_{132} (1). Polyoxometalates with a structure of keplerate were first synthesized about 12 years ago under the guidance of Professor A. Müller (Bielefeld University, Germany). These compounds (Mo_{132}) consist of coordination oxygen polyhedra surrounding ions of molybdenum, as well as stabilizing ligands (acetate ion, etc.), water molecules. Coordination polyhedra by self-assembly in aqueous solutions form up the structure close to spherical, containing a whole 132 molybdenum ion with a charge of 6+ and 5+. In water the resulting compounds form polyanions of about 2.5 nm. A related compound is an iron-molybdenum buckyball $Mo_{72}Fe_{30}$ [5] (2), in which Mo(V) ions are replaced by Fe(III). Researchers' attention was attracted by the internal cavity in keplerates structure and "windows" through which the exchange of water molecules and various substances is possible. Depending on the acidity of the solutions and their concentrations kepleratys decompose into simpler compounds of molybdenum, similar to other known polyoxometalates.

$$(NH_4)_{42}[Mo^{VI}_{72}Mo^{V}_{60}O_{372}(H_3CCOO)_{30}(H_2O)_{72}]\ 30H_3CCOONH_4 \cdot 250H_2O \quad (1);$$

$$[Mo_{72}Fe_{30}O_{252}(CH_3COO)_{12}\{Mo_2O_7(H_2O)\}_2\ \{H_2Mo_2O_8(H_2O)\}(H_2O)_{91}] \approx 150H_2O \quad (2).$$

Molybdenum and iron are essential micronutrients. Biological function of molybdenum is its participation in redox reactions as a cofactor oxidases, including hypoxanthine oxidase, xanthine oxidase, catalyzing the formation of uric acid from purine bases [8,9]. Daily requirement for molybdenum for the man is 0.15 - 0.5 mg, its soluble compounds are easily absorbed in the intes-

*This work was financially supported by the Russian Foundation for Basic Research (Grant 10-03-00799).

tines and does not accumulate in the body. The biological role of iron is related to its participation in the transport and binding of oxygen (hemoglobin, myoglobin) and in redox reactions: heme iron is a coenzyme of cytochromes, catalase, peroxidase, and non-heme iron Fe^{2+} is a cofactor required for oxidation of amino acids and cholesterol. Excess of iron in the body is accompanied by the visceral injury (hemochromatosis), activation of free radical oxidation. Need for iron is 10 - 20 mg/day, but since only 5% - 10% of iron consumed is absorbed [8,9].

Exchange of buckyballs based on molybdenum and iron may be different from the usual exchange of compounds of these elements in the body due to the unique features of nanoflakes, so it is necessary to study the toxicity of buckyballs. Preliminary researches show of complexes formation possibility for buckyballs with a known enough immunomodulator aminophtalhidraside or its derivatives. Aminophtalhidraside promotes regenerative processes in an organism. Therefore we assume further to investigate transport of aminophtalhidraside by intramuscular introduction with buckyballs. Other of possible method of introduction of a complex is also the electrophoresis method.

The purpose of this study: the study of toxicity of buckyballs based on molybdenum and iron, designed as containers or cores for transport of drugs.

2. Materials and Methods

Mo_{132} synthesis was carried out published in [4], and $Mo_{72}Fe_{30}$—by the method [5]. The initial reagents were ammonium heptamolybdate $(NH_4)_6Mo_7O_{24}\cdot 4H_2O$ (chemically pure), hydrazine sulfate $N_2H_4\cdot H_2SO_4$, sodium hydroxide NaOH and sodium chloride NaCl qualification (pure for analysis), ammonium chloride NH_4Cl and hydrochloric acid HCl (purity), ferric chloride (III) $FeCl_3\cdot 6H_2O$ (Panreac, the content of main substance 97 - 102 wt%), acetic acid (chemically pure) CH_3COOH.

Accumulation of buckyballs was investigated during the experiment on 27 rats of both sexes weighing 200 - 230 g, contained on a standard diet of the vivarium. Conditions of housing and treatment to animals corresponded to the EU Council Directive of November, 24 1986 "On the approximation of laws, regulations and administrative statutes of the EU on the protection of animals used for experimental and other scientific purposes "(86/609EES). Animals were divided into 3 groups: 1—intact rats, 2—introduction of molybdenum buckyballs, 3—introduction of iron-molybdenum buckyballs. Previously, it was found that the area of acceptable over time stability of buckyballs is a weak acidic or neutral environment, and the best method of buckyballs introducing—intramuscular injections was chosen [10,11]. Injections were made in the area of the gastrocnemius muscle in a concentration of 10^{-2} mol/L daily for a month. The dose of buckyballs injected corresponded to the upper limit of the normal daily intake of molybdenum and was 21.5 times less than the daily dose of iron. Animals were withdrawn from the experiment by an overdose of ether. Molybdenum content in organs was determined by atomic emissive spectrometer with inductively coupled plasma iCAP-6500 Duo (Thermo Scientific) after mineralization of samples.

Preparation of tissue samples of liver and kidney for histological examination was performed on an automated processor Leica EG 1160, followed by filling in paraffin. Slices of thickness 3 - 5 μm were stained with hematoxylineosin, pirofuchsin by Van Gieson and Weigert. Microscopic examination was performed on a microscope Leica DM 2500, image analysis was performed in VideoTesT "Morphology" 5.0. Photomicrographs of histological specimens were obtained using a digital color camera "CAM 2800".

Analysis of peripheral blood was performed on an automated hematology analyzer Celly 70 Biocode Hycel.

Statistical analysis of the material was carried out using the programs Statistica 6.0 (Stat. Soft.Inc.) and Microsoft Exel 2003. During the statistical hypotheses testing a significance level of 5% ($P < 0.05$) was used.

3. Results and Discussion

In the study of molybdenum content in organs of rats receiving molybdenum buckyballs (group 2), no accumulation of this element in the liver and bones was revealed. In kidneys a sharp decrease in its content by 80% ($P < 0.05$) was revealed. Experiments allowed us to establish the lack of accumulation of molybdenum (**Table 1**) in the liver, kidneys, bones, skin of animals receiving iron-molybdenum buckyballs (group 3). Lack of molybdenum accumulation may be associated with the decomposition of buckyballs into simpler forms that can be easily

Table 1. The molybdenum content (mkg/g in a corresponding part) in rats bodies.

Organ	*Mean content of Mo*	
	Experimental animals	*Intact animals*
Liver	5.3	7.2
Kidney	8.3	12.0
Ossa	1.2	1.8
Cutaneous covering with hair side	3.0	2.9

removed from the body.

Histological analysis of kidney sections of animals from group 2 no structural changes in the glomeruli were detected, but in the part of tubules lumen the eosinophilic mass (**Figure 1**) and focal hyperemia of sinusoidal blood vessels a "sludge-complex" formation were determined.

Histological analysis of kidney sections of animals from group 3, in contrast to group 2, only focal hyperemia of sinusoidal vessels (**Figure 2**) revealed, the tubules epithelium was not changed. The presence of eosinophilic masses and hyperemia of vessels showed sensitization and inflammation in the kidney tubules. These changes are largely present at the animals receiving molybdenum buckyballs.

In the liver of animals from group 2 diffuse hyperemia of central venous and focal hyperemia of the veins of portal tracts were revealed. In the parenchyma of the organ focal hyperemia of sinusoids with the formation of "sludge complex" was noted (**Figure 3**).

In a part of the periportal tracts periportal leukocytic infiltration was found. Increase in the number of Kupfer cells in sinusoids was visually marked. Part of the hepatocytes showed signs of granular dystrophy.

In the liver of animals from group 3 (**Figure 4**), unlike in the liver of animals from group 2, the structural changes of hepatocytes were not observed. Central vein and the vein of portal tracts were plethoric. In some of them the "sludge complex" formation was determined that may be associated with increased blood clotting due to increasing number of platelets and hematocrit index.

Comparing the results of morphometry it was established that index of alteration of the animals from group 2 increased in comparison with the index of intact animals by 13 times, while the figure in group 3 was 3.9

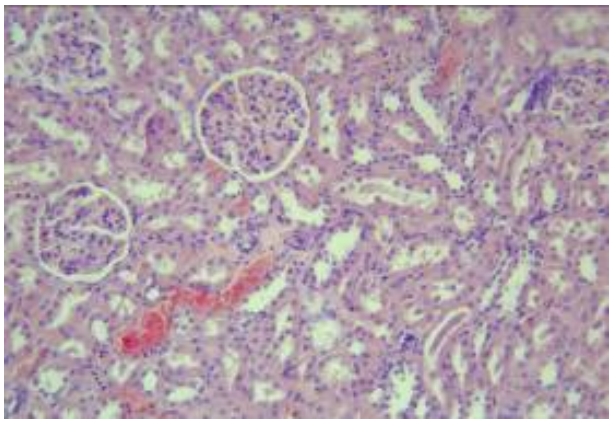

Figure 1. The kidneys of animals of the 2nd group: in the tubules lumen there are eosinophilic masses. Hematoxylin and eosin stain. Magnification ×200.

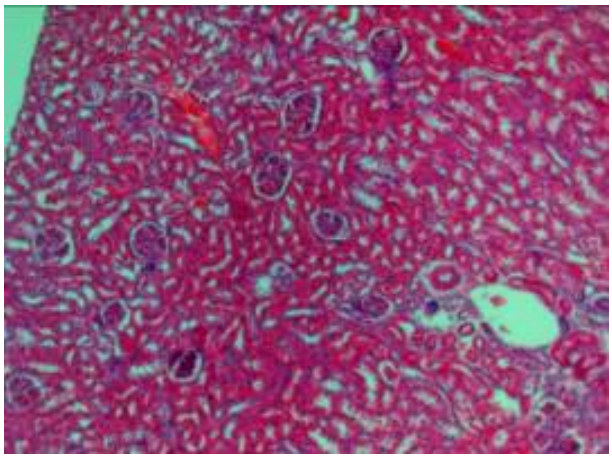

Figure 2. The kidneys of animals of the 3rd group: focal hyperemia of sinusoidal vessels. Hematoxylin and eosin stain. Magnification ×100.

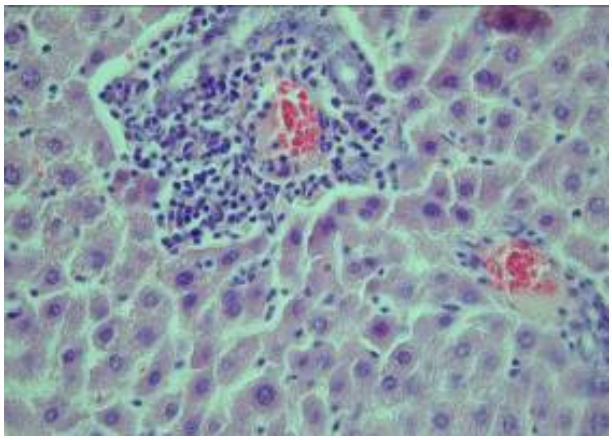

Figure 3. The liver of animals from the 2nd groups: the central vein hyperemia, the "sludge-complex" formation in sinusoids. Hematoxylin and eosin stain. Magnification ×400.

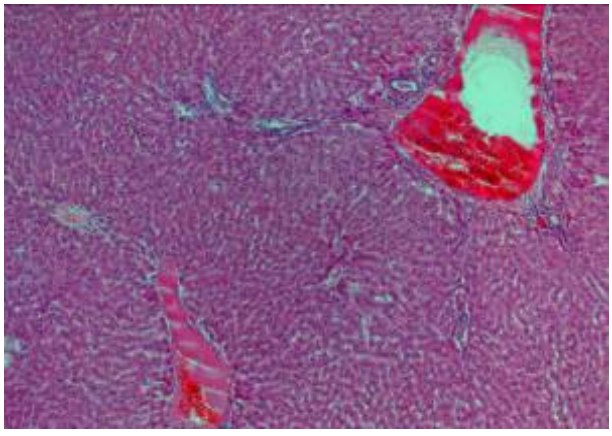

Figure 4. The liver of animals from the 3rd groups: hyperemia of the central vein and the "sludge complex" formation. Hematoxylin and eosin stain. Magnification ×100.

times higher than the control level ($P < 0.05$). Alteration processes were accompanied with activation of the organ's intracellular regeneration, since the number of binucleated cells in the liver of animals from groups 2 and 3, respectively, increased by 1.6 and 1.4 times. Perhaps this is due to an increase in activity and number of sinusoidal cells in the liver. Sinusoidal cells (Kupffer cells, Ito cells) secrete growth factors, cytokines that promote regeneration. An increase in number of sinusoidal cells by 1.5 times in comparison with control was registered for the animals from group 2 only.

Analysis of peripheral blood led to the conclusion that there is no characteristic feature of many chronic intoxications—anemia of animals receiving injections of both buckyballs, as far as hemoglobin in erythrocytes of experimental rats was not reduced. The total number of leukocytes of experimental animals from groups 2 and 3 did not increase, indicating the absence of an inflammatory process of the whole organism.

More pronounced toxic effect of molybdenum buckyballs compared to the iron-molybdenum may occur due to the presence of molybdenum in the oxidation state 5+, involved in renewable redox reactions, and accelerated formation of uric acid and urate intoxication in the renal tubules.

4. Conclusions

1) The toxicity of iron-molybdenum nanocluster polyoxometallates significantly reduced in comparison with molybdenum, which is confirmed by histological studies and analysis of the peripheral blood of animals.

2) The selective cumulativity of buckyballs in the studied tissues was not found, which allows to suggest reversibility of detected changes.

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