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Influence of the mixed-metal Cu/Fe complex $[Cu(dmen)_2][Fe(CN)_5(NO)]$ (dmen=N,N-dimethylethylenediamine) on serum iron and copper levels in experimental anemia of rats

Wpływ mieszanego kompleksu metali Cu/Fe $[Cu(dmen)_2][Fe(CN)_5(NO)]$ (dmen=N,N-dimetyletylenediamina) na poziomy żelaza i miedzi w surowicy krwi w doświadczalnej niedokrwistości u szczurów

INTRODUCTION

It is well known that iron and copper play an important role in red blood cells differentiation. Iron is part of hemoglobin and is necessary for hemoglobin synthesis in red blood cells precursors [8]. Hemoglobin transports oxygen from lungs to cells of various tissues. Copper is part of the proteins that transport and store iron [2, 3, 12, 13, 14]. That is why iron and copper deficiency in the organism causes anemia [4, 7]. The level of these ions can be increased using various medicines, including the novel mixed-metal complex $[Cu(dmen)_2][Fe(CN)_5(NO)]$ (dmen = N,N-dimethylethylenediamine) (code KL447) that has been synthesized at the Taras Shevchenko National University of Kyiv. The complex contains both iron and copper ions. The aim of our study was to investigate the effect of this complex on the dynamics of serum iron and copper in experimental anemia of rats.

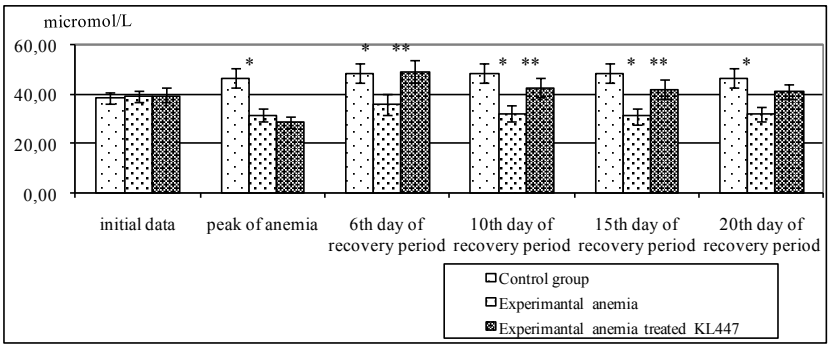
MATERIAL AND METHODS

Experiments were carried out on pubertal age outbred female rats (weight 170–200 g). The rats were divided into three groups: kontrol, experimental anemia and experimental anemia treated with KL447 (eight animals per group). The anemia was caused by the blood loss (1 ml/100 g body weight) 5 times for 10 days. KL447 was dissolved in distilled water and administered *per os* in a daily dose of 25 mg/kg body weight (3 mg/kg Fe(II) and 3.5 mg/kg Cu(II)) since the next day after the last blood

loss during 20 days. The blood was collected from the tail vessels. Serum iron and copper levels were determined using Pliva Lachema's kits and a SUMAL spectrophotometer at the peak of anemia (the next day after the last blood loss), and on the 6th, 10th, 15th and 20th days of the recovery period. Statistical analyses were performed in Excel. The experimental data were normally distributed. Difference between the respective values was statistically significant at $p \leq 0.05$ using Student's t-test.

RESULTS

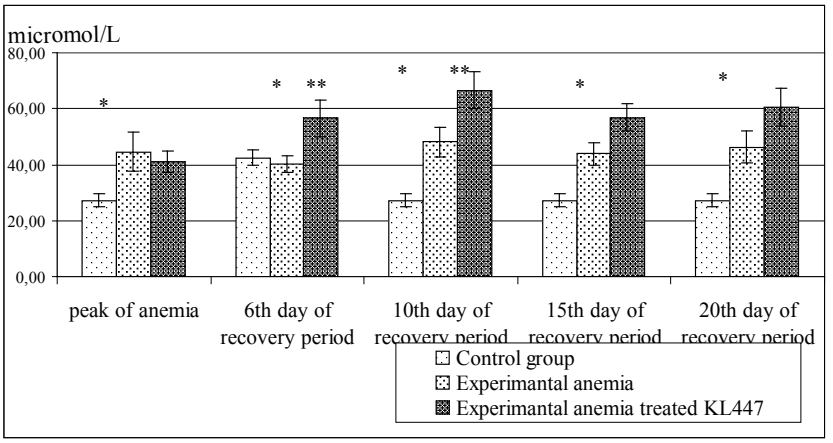
There was no significant difference in serum iron levels between the groups before the experiment (Fig.1). The serum iron levels in the control group revealed a tendency to increase at the peak of anemia in the experimental groups and increased on the 6th day of the recovery period in the experimental groups vs data before the experiment. These changes related to the release of iron ions from storage to be included in erythrocytes recovery (after the blood loss for examination of this parameter). That is why we reduced the blood loss in the control group and compared the last data of iron serum levels in this group with the data of the experimental (anemic) groups on the 10th, 15th and 20th days of recovery period.



* significant difference compared with the control group at $p \leq 0.05$, ** significant difference compared with the group of experimental anemia at $p \leq 0.05$

Fig.1. Influence of mixed-metal complex KL447 on serum iron levels of rats

Serum copper levels were increased in the control group (after the blood loss for examination of this parameter) as well as iron on the 6th day of the recovery period in the experimental groups vs the data at the peak of anemia in the experimental groups (Fig. 2). That is why serum copper levels in the experimental groups were compared with this parameter in the control group at the peak of anemia.



* significant difference compared with the control group at $p \leq 0.05$, ** significant difference compared with the group of experimental anemia at $p \leq 0.05$

Fig. 2. Influence of mixed-metal complex KL447 on serum copper levels of rats

At the peak of anemia, serum iron levels (Fig.1) decreased (31.67±2.47; 28.71±2.55 μmol/l) and the copper level (fig. 2; 44.6±6.97; 41.13±3.75 μmol/l) increased in two anemic groups vs the control group (46.73±3.89; 27.29±2.43 μmol/l, respectively). The serum iron levels were decreased and the copper level was increased during 20 days of the recovery period in the non-treated anemic group vs the control group. KL447 increased serum iron (on the 6th, 10th and 15th days) and copper (on the 6th and 10th days) levels vs the anemic group. The tendency of iron to increase on the 20th and copper on the 15th days of the recovery period retained in the anemic group treated with KL447.

DISCUSSION

Chronic blood loss causes iron defficiency and anemia [5, 9]. In our investigation the blood loss (approximately 10% of the circulating blood volume for five times) in rats results in reduced hemoglobin and serum iron levels. But the serum copper level change in an opposite direction – it increases. These data indicate the role of copper in iron trasport. It is known that copper is part of duodenal enterocytes protein haphaestin that take part in iron absorption [12]. Plasma protein ceruloplasmin (containing copper) plays a role in the mobilization and oxidation of iron from tissue stores with subsequent incorporation into transferrin [3]. Mitochondrial copper-containing proteins frataxin and cytochrome-c-oxidase serve heme synthesis in the erythroblasts [2, 13]. Cu,Zn-Superoxide dismutase catalyses the dismutation of superoxide anions in the cytoplasm and it protects cells against free radicals [14]. That is why the increase of copper serum levels in experimental anemia in the our study can be related to the active synthesis and inclusion of copper in the proteins that participate in the transport of iron. Increase of serum iron levels after treatment with KL447 was shown. KL447 and nitroprusside ($Na_2[Fe(CN)_5NO]\cdot 2H_2O$) have the same anion. Our data correlate with the results of the study nitroprusside influence on serum iron levels in rabbits obtained by

Engeser et al. [1]. Nitroprusside can release iron [11]. It increases heme oxygenase-1 expression and promotes iron regulatory proteins degradation in mouse monocyte macrophage cell line via an increase in intracellular iron [6, 15]. Serum iron levels increase with induction of hepatic heme oxygenase-1 [10]. Moreover, nitroprusside elevates the iron content of the liver more effectively (60%) in comparison with $FeSO_4$ (30%) [1].

CONCLUSIONS

These results indicate that the mixed-metal Cu/Fe complex KL447 causes recovery of the serum iron levels and increases the serum copper levels in the experimental anemia of rats and it could be a potential substance for stimulation of erythropoiesis.

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SUMMARY

The aim of the study was to investigate the effect of novel mixed-metal complex $[Cu(dmen)_2][Fe(CN)_5(NO)]$ (dmen=N,N-dimethylethylenediamine) (code KL447) on dynamics of serum iron and copper in the experimental anemia of rats. At the peak of anemia the serum iron levels were shown to decrease and the copper level – to increase and these retained during 20 days of the recovery period in the anemic group. KL447 increases serum iron and copper levels. These results indicate that the mixed-metal Cu/Fe complex causes the recovery of the serum iron levels and increases the serum copper levels in the experimental anemia of rats and it could be a potential substance for stimulation of erythropoiesis.

Keywords: mixed-metal Cu/Fe complex, serum iron, serum copper

STRESZCZENIE

Celem badań była ocena wpływu nowego kompleksu metali $[Cu(dmen)_2][Fe(CN)_5(NO)]$ (dmen=N,N-dimetylethlenediamina) (kod KL447) na dynamikę żelaza i miedzi w surowicy krwi w przebiegu doświadczalnej niedokrwistości u szczurów. W okresie maksymalnego nasilenia anemii stężenie żelaza w surowicy było obniżone, zaś miedzi – podwyższone i stan ten utrzymywał się przez kolejne 20 dni okresu zdrowienia. KL447 zwiększał poziomy żelaza i miedzi w surowicy. Wyniki przeprowadzonych badań wskazują, iż kompleks KL447, przyczyniając się do wzrostu poziomów Fe i Cu w surowicy krwi, może być potencjalną substancją wykorzystywaną do stymulacji erytropoezy.

Słowa kluczowe: mieszany kompleks metali Fe/Cu, miedź, żelazo, surowica