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The Effect of Erythropoietin on Rat's Red Blood Cell Indices in Simulated Microgravity (Experimental Study)

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ABSTRACT

Purpose: Microgravity causes major changes in various systems of the body in space, such as shift in cerebrospinal fluid, decreased red blood cells count, and electrolyte loss. These complications are very important in space and we should find new approaches to prevent the side effects of microgravity in astronauts.

Materials and Methods: This experimental study was conducted on 21 adult male rats in three groups: control, Hind-limb unloaded, Hind-limb unloaded plus Erythropoietin. SPSS software was used for data analysis. RBC indices were assessed in the first, third and fourteenth day in different groups.

Results: The highest mean of hemoglobin was 17.98 ± .35 in the Hind-limb unloaded plus Erythropoietin group (on the 3rd day) and the lowest amount was 13.52 ± 1.22 in the Hind-limb unloaded group (on the 14th day). The *P* value to compare RBC and reticulocyte count in Hind-limb unloaded group with those in Hind-limb unloaded plus Erythropoietin group was .017 (on the3rd day), to compare hemoglobin in Hind-limb unloaded group with that in Hind-limb unloaded plus Erythropoietin group was 0.004 (on the 3rd day), and to compare reticulocyte values in Hind-limb unloaded group with those of Hind-limb unloaded plus Erythropoietin group was 0.036 (on the 14th day).

Conclusion: The lowest amount of RBC indices was in the Hind-limb unloaded group (on the 14th day). RBC indices were significantly higher in Hind-limb unloaded plus Erythropoietin group than those on the 1st day. Erythropoietin injection induced significant improvement in RBC indices in rats under microgravity condition. Erythropoietin is very useful to prevent space anemia and its highest effect occurson the 3rd day after injection. This is as an innovative method to prevent space anemia.

Keywords: microgravity; anemia; erythropoietin.

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INTRODUCTION

Microgravity is a condition in which people or objects appear to be weightless, and it induces changes in the physiology and function of living organisms. Microgravity causes major changes in various systems of the body in space, such as cerebrospinal fluid shift, red blood cells (RBCs) count decrease, electrolyte, muscle, mass and bone loss, immune response suppression, change in gastric emptying and intestinal motility and change in liver metabolism. Today, we can detect several types of physiological changes in living organisms by medical imaging techniques for the purpose of spaces

research.[1] Bone mass reduction is due to reduced calcium uptake and body weight pressure. In Apollo space mission, several viral and bacterial infections were occurred due to immune deficiency one week after space travelling.[2] Microgravity induces RBCs Hemolysis and plasma volume reduction. Red blood cells membrane plays an important role in cell resistance against various stresses such as gravity changes and Hypothermia.[3] Microgravity reduces the number of circulating RBCs and plasma volume about 15 percent.[4] Plasma volume decreases due to overall body water and central venous pressure reduction.[5] Orthostatic intolerance is more severe in a long space travelling duration.[6] Increased synthesis of red blood cells in microgravity conditions, improves quality of life in astronauts.[5] Microgravity causes major changes in various systems of the body[7] such as fluid shifts andosteoporosis,[8] it also affects cardiovascular and autonomic nervous system due to decreased blood volume.[9] The Changes in the autonomic nervous system have several effects on the cardiovascular function in space. The above mentioned reasons, are very important to make useof new approaches to control the effects of microgravity on astronauts by drugs.[10] Erythropoietin deficiency is one of the main causes of mortality and morbidity in developing countries.[11] Human recombinant erythropoietin is useful to treat anemia, it also causes an increase in the count of reticulocyte, hematocrit (HCT) and transferrin receptors.[12] Microgravity simulation is conducted in space-based technology laboratories that are unique and have excellent approach to study the cellular biochemistry and regulating metabolic pathways.[13] Rats are the best option for space biology research since they have genetic similarity with human. The purpose of this study is to investigate the effects of microgravity on RBC indices and erythropoietin vestigial on space anemia and its complications.

MATERIALS AND METHODS

This study was approved by the Ethics Committee of Army University of Medical Sciences. At first, we searched about the sustenance condition of rats in microgravity, their characterization, habitat requirements and cage design principles to respect animal rights. We provided standard conditions to induce microgravity and provided their food, water and hygiene. We gathered 21 adult male Wistar rats (three months of age and average weight of 250 g). All rats had similar characteristics at the beginning of study. We assigned a number to each rat and entered their number into the computerized list and divided them to 3 groups, randomly.

The rats had the same conditions (12-12-hour light and dark cycle, humidity of 60% ± 10%, temperature of 23° ± 2° C, food & clean water ad libitum). For the best adaptation, they were kept together a week before commencement of the study.

The first group (control group) was kept in cages without tail suspension, and the second group, Hind-limb unloaded (HU) along with the third group, HU with Erythropoietin injection (HU+ E) were kept for 14 days.

Recombinant human erythropoietin was injected (300 IU/kg) subcutaneously every other day until the 14th day in the third group. Erythropoietin trademark was EPOLYREC® (recombinant human erythropoietin in Iran).

The blood sampling was conducted in the 1st, 3rd and 14th day of the study. All samples were sent with specific numbers for analysis. The lab technicians who were responsible for data and blood analysis, were blinded to rat groups. At first, all rats were anesthetized with intraperitoneal injection of ketamine (50 mg/kg) and xylazine (.1 mg/kg). Then, their tail was disinfected (1 cm from the trunk tail junction) with alcohol 70%, and was perforated horizontally with a 20G sterile needle with precaution to prevent animal's tail vein damage. Then we used a 30 cm stainless steel wire into the needle, and performed a ring to suspend the rats from the cage roof (hind-limb unloading). For blood sampling purposes, animals were anesthetized with ether. Blood sampling was conducted from vena cava.

We collected blood samples in the 1st, 3rd and 14th day of the study. Cardiac puncture was used for blood sampling in the last day. Hemoglobin (HGB), reticulocyte, and RBC count were measured in this study. Data was entered in SPSS version 22 to calculate frequency distribution, central tendency and dispersion. Wilcoxon Signed-Rank Test was used to compare the two related samples in different days (before-after) and Mann-Whitney *U*test wasused to compare the two independent groups in the same days.

RESULTS

In all three groups, the descriptive analysis of blood parameters had equal ranges at the 1st day of study. All rats were in similar condition, were included in the statistical analysis and were evaluated from beginning to the end of study. No missing data was there during this research.

The highest mean of, HGB, RBC and reticulocyte count was in the HU+E group on the 3rd day while the

lowest amount of those, was in the HU group on the 14th day (**Table 1** to **3**). RBC indices values are fully descripted in **Table 1** to **3**. The changes of RBC indices are shown in **Figure 1** to **3**.

Wilcoxon sign test was used to compare the amount of RBC indices in the two dependent groups in different days (before-after). The *P*value to compare HGB, Reticulocyte and RBC values between the 1st day of the study and the 3rd was .028.

Mann-Whitney *U* test was used to compare the amount of RBC indices in the two independent groups in the same day. The *P*value tocompare HGB, RBC, and Retic counts between HU and HU+E groups was .004, .017, and .017, respectively,on the 3rd day.

Table 1. Hemoglobin descriptive data in various stages of research

MEAN ± SD	Max	Min	Group /Day
15.99 ± .76	16.94	14.93	Control / 1st day
15.97 ± .76	16.92	14.91	Control / 3rd day
15.95 ± .75	16.9	14.9	Control / 14th day
15.97 ± .75	16.93	14.92	HU+E / 1st day
17.98 ± .35	18.4	17.6	HU+E / 3rd day
16.43 ± .94	17.5	15.7	HU+E / 14th day
15.98 ± .77	16.92	14.91	HU / 1st day
16.42 ± .86	17.6	15.4	H / 3rd day
13.52 ± 1.22	14.6	12.1	HU / 14th day

Table 2. RBC descriptive data in various stages of research

MEAN ± SD	Max	Min	Group /Day
8 ± 0.72	9.18	7.26	Control / 1st day
8.1 ± 0.7	9.2	7.25	Control / 3rd day
8 ± 0.72	9.18	7.26	Control / 14th day
8.1 ± 0.71	9.17	7.25	HU + E / 1st day
9.19 ± 0.49	9.65	8.27	HU + E / 3rd day
8.35 ± 0.3	8.57	8	HU + E / 14th day
7.8 ± 0.7	9.16	7.25	HU / 1st day
8.25 ± 0.88	9.08	6.89	HU / 3rd day
6.9 ± 0.08	7.85	6.11	HU / 14th day

Table 3. Reticulocyte descriptive data in various stages of research

MEAN ± SD	Max	Min	Group /Day
0.51 ± 0.83	2.2	0	Control / 1st day
0.5± 0.8	2.1	0	Control / 3rd day
0.51 ± 0.83	2.2	0	Control / 14th day
0.52 ± 0.82	2.3	0	HU+E / 1st day
2.16 ± 1.44	4.8	0.57	HU+E / 3rd day
2 ± 1.8	4	0.5	HU+E / 14th day
0.5 ± 0.81	2.1	0	HU / 1st day
0.32 ± 0.41	0.93	0	HU / 3rd day
0.138 ± 0.121	0.33	0	HU / 14th day

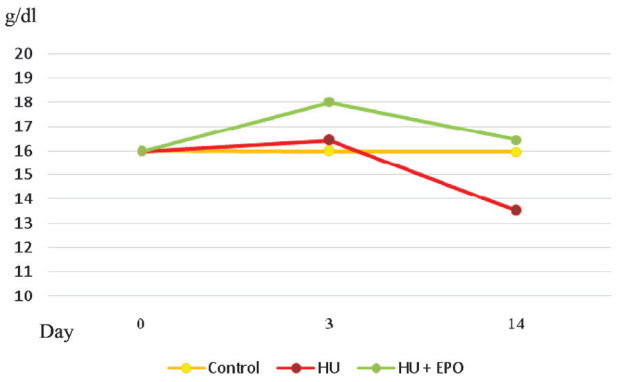

Chart 1. Hemoglobin changes in three stages

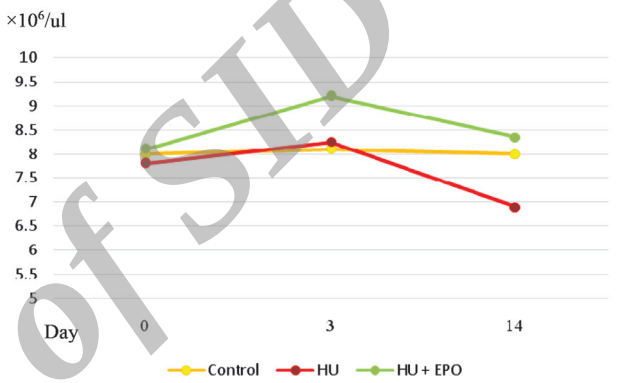

Chart 2. RBC changes in three stages

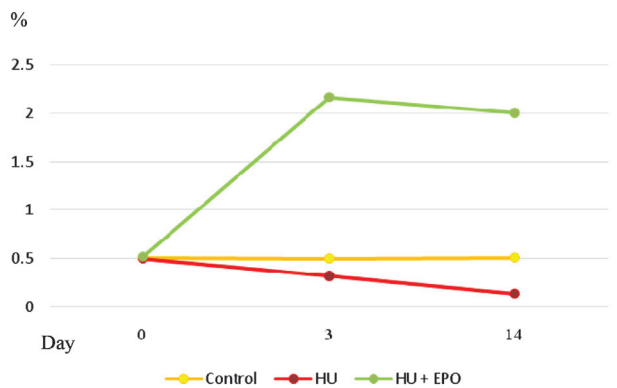

Chart 3. Reticulocyte changes in three

DISCUSSION

This study investigated the effects of microgravity and erythropoietin on rat RBC indices.The Lowest amount of blood indices was seen in the HU group at the end of the study (14th day) because microgravity induced anemia in rats with bone marrow suppression and reticulocyte reduction.In the same studies, microgravity effects were investigated in rats and anemia was found due to erythropoietin deficiency and bone marrow suppression.[14-16]

The rats' RBC indices have been investigated in various studies.[17] Three days after erythropoietin injection, HGB, RBC and Retic counts began to rise and reached to their maximum amount in the HU+E group.The major effect of erythropoietin is on the bone marrow & progenitor cells.[21,22] We found there is a significant difference between RBC indices on the 3rd and the 1st day (under microgravity condition). Exactly, the results were better after erythropoietin injection on the 3rd day.Erythropoietin had an effective role in bone marrow hematopoiesis up to the third day in microgravity condition. Also, there is significant difference between RBC indices in HU and HU+E groups. The values were greater after erythropoietin injection.

Erythropoietin had been assessed in anemia treatment without microgravity conditions in previous studies. Our innovation was using erythropoietin injection at the beginning of study for prevention of space anemia and we found its prominent effects until the 3rd day.

RBC indices increased significantly up to the 3rd day and then decreased, because the bone marrow did not have more capacity to produce more RBC. However, the amount of RBC indices was upper on the 14th day in comparison with the first day in rats with erythropoietin injection.

In the HU group, no statistically significant difference was found in RBC indices before and after of microgravity due to the low number of rats in HU group, but a clinically significant decrease was foundin RBC indices in this group.

Researchers have found a significant decrease in RBC indices after microgravity induction in higher sample sizes. [23] In a similar study with simulated microgravity, a significant decrease was found in HGB. [24]

In this studya significant increase was found in the RBC indices on the third day in HU+E group. However, there was no significant difference in RBC indices in the on 14th day due to the reduced capacity of bone marrow in HU+E group.

In a similar study increased blood parameters was seen after erythropoietin injection in rats.[25]It is known that erythropoietin deficiency is one of the main cause of anemia in weightlessness.[26] Microgravity reduces the level of erythropoietin and causes bone marrow suppression and low sympathetic stimulation.[27]

In another similar study, patients with impaired sympathetic system and erythropoietin deficiency had severe anemia. Their condition was improved after recombinant human erythropoietin injections (50 IU/kg) three times a week. [27]Nevertheless, nobody has studied the effect of erythropoietin in microgravity. [18,28]

Bone marrow suppression, impaired homeostasis, differentiation, migration and proliferation of blood cells are the aspects appearing in microgravity.[30]Numerous studies have shown that erythropoietin deficiency can be compensatedby an injection.[27,29] In our study, adecrease was seen in RBC parameters after the 3rd day due to reaching to the highest capacity of bone marrow.

CONCLUSIONS

Erythropoietin is effective in the treatment of space anemia.Although RBC indices decreased, after the 3rd day, due to the maximum bone marrow response to erythropoietin, it is worth mentioning that values maintained at a higher level compared with the 1st day.

Strengths and Limitations

The strength of this study was its novelty in erythropoietin injection in microgravity condition. The most important limitation was designing and building new standard cages for microgravity induction in rats. Metabolic cages did not have enough standards for research in this field.

Recommendations

We suggest to produce more standard cages toinduce weightlessness in rats in order to improve the statistical power and to gain the best conclusion.

CONFLICT OF INTEREST

None declared.

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