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Research Article

Ghrelin Increases Lymphocytes in Chronic Normobaric Hypoxia

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Abstract

Purpose: Hypoxia is a condition of decreased availability of oxygen. To adapt hypoxia, some changes in blood cells occur in the body. The aim of this study was to evaluate the effect of ghrelin on different types of blood cell in normobaric hypoxia situation.

Methods: Thirty-two animals were divided in 4 groups (n=8): control (C), ghrelin (G), hypoxia (H), and hypoxic animals that received ghrelin (H+G). Hypoxia (11%) was induced by an Environmental Chamber System GO2 Altitude. Animals in ghrelin groups received a subcutaneous injection of ghrelin (150 µg/kg/day) for 14 days.

Results: Our results show that ghrelin significantly ($p<0.05$) increased RBC and Hct levels, whereas it significantly ($p<0.05$) decreased lymphocytes in the blood. RBC, Hct, Hb concentration, platelet and MCV increased significantly ($p<0.05$) in hypoxic conditions but lymphocytes, monocytes and Polymorphonuclears did not show any significant changes. Platelets had a significant ($p<0.05$) decrease in hypoxic conditions and ghrelin administration in hypoxic conditions could increase lymphocyte levels significantly ($p<0.05$).

Conclusion: Effect of ghrelin on blood cells could be related to blood oxygen level. Ghrelin in normal oxygen conditions increases RBC and Hct levels but decreases lymphocytes, whereas in hypoxic conditions, ghrelin increases blood lymphocytes.

Introduction

Hypoxia, a condition of decreased availability of oxygen, contributes to the regulation of pathophysiology in various kinds of cells and tissues.[1] When a cell or an organism is in an abnormal condition starts series of mechanisms to adapt or response at the cellular and molecular levels as strategies to minimize serious effects of the condition.[2] To adapt hypoxia, the cell should reduce energy consumption or increase oxygen supply by inducing a change in red blood cells.[2,3] Other blood cells including white blood cells and platelets also undergo some changes in hypoxic conditions.[4-6] However, accomplished studies in hypoxic conditions demonstrated that hypoxia causes reduction in proliferation of lymphocytes.[4] Whereas, another study has shown that hypoxia increases blood lymphocytes.[7] One study done on the effects of hypoxia on platelet indicated that increase in altitude could reduce platelets.[8] Another study showed that hypoxia, in the early-onset, causes thrombocytosis, however, in the late-onset, it causes thrombocytopaenia.[6,9]

In addition to hypoxia, endocrine factors such as growth hormone and glucocorticosteroids can also affect the production of blood cells.[10,11]

Ghrelin is a 28-amino acid peptide hormone that is considered by researchers for its physiological effects. It is found in the secretory granules of X/A-like cells in gastric mucosa.[12] Today, studies have shown that ghrelin is also produced by other tissues such as kidney, pancreas, placenta, testis, pituitary, lung, and hypothalamus.[13,14] The acylated form of ghrelin has a serum half life of only 30 minutes because of rapidly change to a deacylated form that is more stable. Acylated ghrelin binds to the growth hormone secretagogue-receptor1a (GHSR-1a) in many tissues to produce its effects.[15] Among the known physiological actions of ghrelin are; glucose homeostasis, growth hormone secretion, appetite stimulation and adipogenesis, cell proliferation and survival, increase in GI motility.[13,15] It has also anti-inflammatory, cardiovascular, sleep regulation, and reproduction effects.[13,15-17]

In studies examining the impact of the ghrelin on blood cells we faced with contradictory results. One study has shown ghrelin has no effect on red blood cell (RBC), hemoglobin (Hb) concentration, and hematocrit (Hct),

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mean corpuscular volume (MCV).[18] But, another study has shown that ghrelin increases RBC, Hct and decreased MCV.[19] Several studies have demonstrated that ghrelin dose dependently inhibits the proliferation of lymphocytes and expression of cytokines, while another study has reported an increase in blood lymphocytes.[18,20-22] It has also been reported that ghrelin increases monocytes, eosinophils and basophils.[20] Whereas, Narin et al showed ghrelin decreases neutrophils and it has no effect on monocytes, eosinophils and basophils.[18]
Therefore, in the present study we decided to investigate the influence of ghrelin on blood cell types in hypoxia.

Materials and Methods

Animals and chronic hypoxic protocol

All experiments were performed in agreement with guidelines of the Tabriz University of Medical Sciences for care and use of laboratory animals. Male adult Wistar rats (200–250 gr) were housed in cages in a temperature- and light-controlled environment. Food and water were available ad libitum. Animals were randomly divided in 4 groups (n=8) including control (C), ghrelin (G), hypoxia (H), and hypoxia with ghrelin (H+G). In hypoxic groups (H and H+G), hypoxia (O2 11%) was induced by an Environmental Chamber System GO2 Altitude (Biomedtech Australia, Pty. Ltd). Animals were kept in the chamber continuously for two weeks except for 20 min/day to clean the cages and perform daily injections.

Drug administration

Ghrelin was obtained from the Tocris Bioscience Co. (Bristol, UK). Rats in ghrelin groups (G and G+H) received a subcutaneous injection of ghrelin (150 µg/kg/day).[23] G and H+G rats continued to receive daily injections of ghrelin for 2 weeks.

Blood cells count, hemoglobin and hematocrit measurement

Animals were deeply anaesthetized with ketamine (100mg/kg) and trunk blood was collected immediately after decapitation of the animal. Then, RBC and WBC count were performed. Hemoglobin (Hb) concentration and hematocrit were measured using cyanmethemoglobin and microhematocrit methods, respectively.

Statistical analysis

Results are reported as Mean±SEM. Data were analyzed by ANOVA to test for differences between groups. For statistically significant comparisons, post-Hoc analyses were performed using Tukey tests and $P<0.05$ were used as the level of significance for all statistical analyses.

Results

Effects of hypoxia and ghrelin treatment on RBC, hemoglobin concentration and hematocrit

There was a significant ($p<0.05$) increase in RBC level in Ghrelin group (G) compared to the control group(C). Also hypoxia (H) and hypoxia + ghrelin groups (H+G) had significant ($p<0.05$) increase in RBC level compared to control. Hematocrit in the G group was significantly ($p<0.05$) more than the C group. There was also a significant difference ($p<0.05$) between H+G, H and control groups. Hemoglobin concentration showed no significant difference between groups C and G. There were significant ($p<0.05$) increase in hemoglobin concentration in H and H+G groups compared to C and G groups. Whereas there was not a significant difference between groups H and H+G. But H+G group had not significant difference in RBC, hematocrit and hemoglobin concentration compared to H group.
Hypoxia and H+G groups had significant ($p<0.05$) increase in MCV compared to control group. While, there were no significant differences in MCV either between control and ghrelin or H and H+G groups (Table 1).

Table 1. Effects of hypoxia and ghrelin treatment after 2 weeks on RBC, Hb, Hct and MCV

Groups	Control	Ghrelin	Hypoxia	Hypoxia + Ghrelin
RBC (× 10^6)	5.4167±0.22	6.018±0.08*	6.81±0.17*	6.97±0.08*#
Hb (g/dl)	13.66±0.17	14.73±0.22	22.07±0.29*	22.53±0.54*#
Hct (%)	39.33±0.88	45.66±0.61*	67.14±0.51*	67.87±1.32*#
MCV (fL)	72.72±1.35	75.91±1.05	98.62±0.57*	97.44±0.83*#

Red blood cell (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean Corpuscular Volume (MCV). Data are expressed as mean ± SEM for 8 animals. * $p<0.05$ vs the control group, # $p<0.05$ vs the ghrelin group.

Effects of hypoxia and ghrelin treatment on polymorphonuclears, mononuclears and lymphocytes counts

There were no significant differences in intergroup comparisons in polymorphonuclear and monocyte counts (data are not shown). Ghrelin could significantly ($p<0.05$) reduce lymphocytes compared to control group. Neither hypoxia nor H+G groups did show significant difference in lymphocyte count compared to control group. Hypoxia group that received ghrelin showed a significant ($p<0.05$) increase in lymphocyte level compared to hypoxia group (Figure 1).

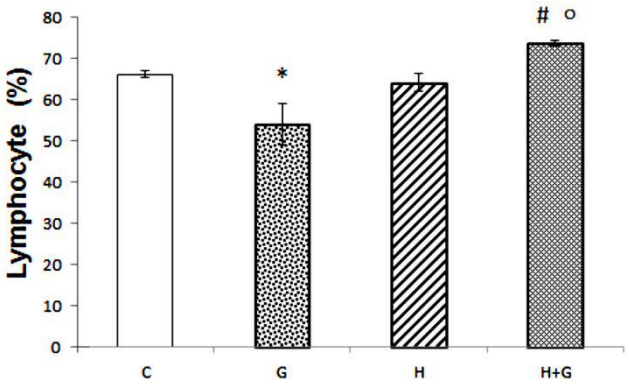

Figure 1. Effect of ghrelin on lymphocyte after 2 weeks in control (C), ghrelin (G), hypoxia (H) and hypoxia plus ghrelin (H+G) groups. Data are expressed as mean ± SEM for 8 animals.* p<0.05 vs the control group, # p<0.05 vs the ghrelin group, ○ p<0.05 vs the hypoxia group.

Effects of hypoxia and ghrelin treatment on platelet count

There was not a significant difference between control and ghrelin groups. Blood platelets decreased in both hypoxia and H+G groups significantly ($p<0.05$), although the difference between H and H+G groups was not significant (Figure 2).

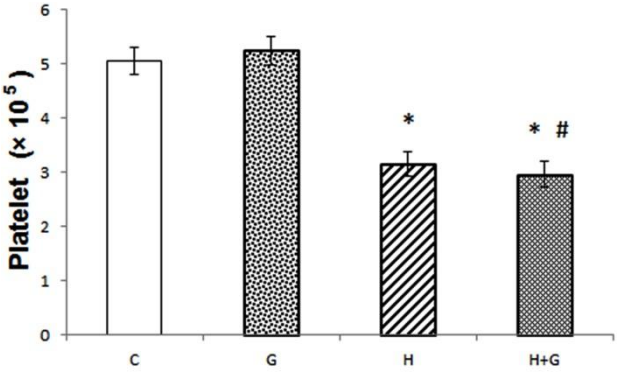

Figure 2. Effect of hypoxia and ghrelin on Platelet after 2 weeks in control (C), ghrelin (G), hypoxia (H) and hypoxia plus ghrelin (H+G) groups. Data are expressed as mean± SEM for 8 animals. * P< 0.05 vs the control group, # p<0.05 vs the ghrelin group.

Discussion

Our study showed that ghrelin increases RBC and Hct levels, whereas it decreases lymphocytes. RBC, Hct, Hb, MCV increased and platelets decreased in hypoxic conditions but lymphocytes, monocytes and polymorphonuclears did not show any significant changes. In hypoxia conditions, ghrelin treatment could increase lymphocytes but had no effect on other parameters.

Taati et al.'s study showed that ghrelin increases RBC and Hct, decreases MCV but it has no effect on Hb concentration.[19] Some studies have suggested that ghrelin directly affects cell division and replication in bone marrow.[19,24] Stimulation of the growth hormone secretion is the most known action of the ghrelin.[15] Ardizzi et al. also demonstrated that growth hormone has a stimulatory effect on erythropoietin in mammals.[25]

Akarsu et al. stated that there was a positive relation between Hb concentration and peripheral ghrelin level. They demonstrated in their study that patients with iron deficiency anemia had low peripheral ghrelin levels.[26] Vander Lely et al. also suggested that low blood ghrelin levels could be the cause of anemia.[27]

Our results about RBC, Hct and Hb concentration are consistent with Taati's study but according to Hb concentration it is not compatible with Akarsu et al.'s study.

Narin et al. in their study showed that ghrelin does not affect RBC, Hb and Hct.[18] Aghdam et al. also suggested that intracerebrovascular injection of 0.5 or 1.0 mg ghrelin/kg at 21 days of age did not have any significant effect on the measured erythropoietic indicators including RBC, Hb, Hct, MCV, MCH and MCHC.[28] Narin et al and Aghdam et al.'s findings are inconsistent with our study. These differences can be attributed to the type of animal, method and dose of drug administration, and time interval between treatment and sampling.

Taati et al. has pointed that ghrelin has no effect on lymphocytes, Whereas, Narin et al. reported increased lymphocyte.[18,19] Other studies showed that ghrelin dose dependently has positive increasing effect on polymorphonuclears and decreasing effect on lymphocytes.[20,21] It should be noted that all the above studies were performed in the normal oxygen conditions.

Szigligeti et al.'s study showed that hypoxia decreases lymphocytes proliferation.[4] Wang et al. in their study suggested that 12% hypoxia increases entry lymphocytes to blood.[7] They expressed it could be due decreased antioxidants and stress oxidative.

Hypoxia induces oxidative stress in blood and leads to aging and apoptosis of lymphocytes.[7] Hypoxia also inhibits voltage-dependent potassium channels (Kv1.3). These channels are essential for the activation and proliferation of lymphocytes.[4] Therefore hypoxia could reduce the number of lymphocytes.

Ghrelin acts as an antioxidant in various tissues such as ovary, stomach, kidney, and neurons.[29-32] It also has antiapoptotic effects in hippocampus, adrenal tumor and cardiovascular system.[33-35]

A significant increase in lymphocyte in hypoxia despite receiving ghrelin may be attributed to the effect of ghrelin on decreasing oxidative stress and apoptosis also its effect on Kv1.3 channels. Thus it is noted that the environmental conditions such as hypoxia is effective on the activity of lymphocytes and the production and secretion lymphokin and probably ghrelin effect.[5] But the proof of this hypothesis requires further investigation.

About platelets, Bradford in his study showed that hypoxia has the dual effect on platelets.[6] The early-onset thrombocytosis caused by hypoxia may be due to increased release of platelets from megakaryocytes and the late-onset thrombocytopaenia may be due to decreased platelet production and/or stem cell competition between erythrocytes and megakaryocytes.[6] Lehmann et al. showed high-altitude leads to platelet

aggregation, platelet consumption, and decreased platelet count.[8] Mc Donald et al. showed long term hypoxia (6-7 days) causes decreased platelets counts and short term hypoxia (1-3 days) increased it which is in agreement with our results.[9]

Conclusion

Effect of ghrelin on blood cells could be related to blood oxygen level. Ghrelin in normal oxygen conditions increases RBC and Hct levels and decreases lymphocytes, whereas, in hypoxia ghrelin increases blood lymphocytes.

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Ethical issues

The study protocol was designed in accordance with NIH guidelines and Ethics Committee for the Use of Animals in Research at Tabriz University of Medical Sciences.

Conflict of interest

The authors have declared that there is no conflict of interest.

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