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Glutamate Agonists May Affect the Hematological Profile in Healthy Rats

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Authors' contributions

This work was carried out in collaboration between all authors. Authors AX, MA and VG designed the study. Authors AX, DK and MK-K performed the statistical analyses. Authors AX and MA wrote the protocol and wrote the first draft of the manuscript. Authors AX and DK managed the literature searches. Authors DK and MK-K managed the analysis of the study. All authors read and approved the final manuscript.

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ABSTRACT

Aims: Even though glutamate is one of the primary endogenous amino acids of the Central Nervous System (CNS) its subunit receptors exist also in non-neuronal tissues outside CNS such as the hematopoietic system. The purpose of this paper is to define the possible *in vivo* effect of glutamate ionotropic agonist Monosodium l-glutamate (MSG) in the hematopoietic system of Wistar adult rats.
Methodology: MSG was administrated intravenously in male Wistar rats of 250-350g weight. Animals treated with MSG (n = 24) were compared to a control group (n = 10). Full blood count with differential, aggregation intensity and bone marrow cellularity were evaluated 12 and 24 hours after drug administration. The results were analyzed by unpaired t-test.

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Results: MSG showed to affect white blood cell count in a negative way whereas it provoked an increase in Hemoglobin (Hb) and Hematocrit (Hct) levels. Aggregation was only transiently affected using ADP as an agonist and bone marrow counts showed a trend towards normalization.
Conclusion: It is concluded that MSG can affect the hematological profile and bone marrow cellular composition of healthy intact rats as well as blood elements functions. Our results suggest a possible role for glutamate receptors on the hematopoietic system's pathophysiology. Further research is needed in order to better characterize the *in vivo* effect of glutamate receptors agonists and antagonists on blood elements and bone marrow.

Keywords: Monosodium l-glutamate; glutamate; glutamate receptors; blood; bone marrow.

1. INTRODUCTION

Glutamate is one of the main endogenous CNS amino acids that along with its receptors is widely known to play an important part in synaptogenesis [1], learning and memory [2], Alzheimer's disease [3] and epilepsy [4].

Based on their pharmacological and physiological properties Glutamate receptors can be divided into two categories:

1. Ionotropic receptors, named after their agonists, N-methyl-D-Asparate (NMDA) [5], A-amino-3-hydroxy-5-methyl-4-isoxazolopropionic acid (AMPA) and kainate acid.
2. Metabotropic receptors connected with intracellular messengers [6].

Glutamate receptors have been found to be present in many peripheral tissues including adrenal medulla [7], peripheral nerves, myelinated and unmyelinated [8], bone [9], endocrine pancreas [10], esophagus [11], hepatocytes [12], heart [13,14], taste buds [15,16], keratinocytes [14], lungs [17], pituitary [18], pineal gland [19], ileal longitudinal muscle [20], autonomic and sensory ganglia [7], kidney, spleen, ovaries [11] and stomach [21]. Last but not least, glutamate receptors have been found in peripheral blood elements and in bone marrow cells (as mentioned above) suggesting that glutamate can act as a widespread cytokine despite tissue location.

Monosodium glutamate is used as flavoring of foods [22]. MSG was considered as a potential migraine headache and asthma triggers or that causes a variety of symptoms known as the "Chinese restaurant syndrome" but there are no consistent data to support this relationship [23]. Furthermore, earlier studies have demonstrated that exposure to MSG causes neuroendocrine abnormalities and leads to obesity, nociception, impairment of memory, anxiogenic-like and depressive-like behaviors [24-26].

To focus on the hematopoietic system, glutamate receptors have been identified in megakaryocytes, platelets and lymphocytes [27,28].

Research proved the presence of NR1 and NR2D subunits in human and rat megakaryocytes as well as in MEG-01 cell line [29]. In human megakaryocytes, there are also NR2A subunits as well as Yotaio and PSD-95 helping proteins [30]. Yet, the absence of proteins related to the CNS NMDA receptor suggests than even though those receptors seem to be similar they are definitely not identical.

Platelets have been described to alter their function in the presence of NMDA and glutamate [31,32]. Fanconi et al. [33] proved that glutamate has an anti-aggregating activity on platelets primarily exposed to arachidonic acid or ADP or PAF leading to the conclusion that platelet NMDA receptors have a selective affinity to their agonists. Later on, NMDA receptor activation (either by NMDA or by glutamate, the first being 3 times more powerful than the later) has been found to antagonize the aggregating activity of arachidonic acid in human rich platelet plasma while there is no such effect neither from NMDA nor from glutamate. NMDA had no effect in the u-46619 induced aggregation, excluding glutamate action from any relation with TxA2 / PGH2 receptors on platelets. Furthermore, platelets have glutamate transporters that release glutamate upon their activation [34].

Kostayan et al. [35] proved the presence of glutamate binding sites on the surface of human lymphocytes. These studies were expanded in rodents. It has been found that there are subgroups I and II of metabotropic receptors in thymus, isolated thymus cells, Thymic stromal cell line [36]. Subgroup III of metabotropic

receptors and NR1 subunit of NMDA ionotropic receptors can be found in recently isolated lymphocytes [37].

In humans, GluR3 subunits of AMPA ionotropic receptors have been found on peripheral T-Cells, in Jurkot T leukemic cell line and in a CD4 alloprimed T-helper clone [38]. Group I of metabotropic receptors has also been found in both active and non-active T-cells as well as in several lymphoid series [39]. Miglio et al. [40] have suggested the expression of subunits NR1 and NR2 genes in human cells. In contrast with NMDA receptors in megakaryocytes, NMDA receptor in human lymphocytes was found to be similar to the CNS receptor. To date, it has been found that glutamate in direct interaction with its AMPA receptors triggers integrin-mediated adhesion to laminin and fibronectin, just like activated cells.

No specific glutamate receptors subtypes have been described for erythrocytes although they have been described to contain pools of glutamate.

Yet, even though all of the above suggest that glutamate receptors play an important role on T-cells, platelets and bone marrow megakaryocyte function there are grey zones to our knowledge concerning the possible in vivo effect of glutamate receptors and their agonists in hematopoietic diseases.

In order to address this issue, we hereby investigate the clinical effect of glutamate receptor agonist MSG on the hematopoietic system of healthy intact Wistar rats using routine lab tests.

2. MATERIALS AND METHODS

2.1 Animals

Study group consisted of 34 male Wistar rats (weighting about 300g each). They were housed in separate cages, kept under the same conditions of temperature (22±2ºC and humidity) and were fed on a standard diet. Access to tap water via water bottles was ad libitum.

Rats were anesthetized with chloral dehydrate 4,5% 1 ml/100 gr BW. Rats after anesthesia removed from the induction chamber after loss of righting reflexes, and anesthesia was maintained by using ether (it was necessary) through a diaphragm-covered nose cone. Heart rate and hemoglobin oxygen saturation were monitored (Pulse Sense VET Portable Tabletop Pulse Oximeter, Nonin Medical, Plymouth, MN).

2.2 Drug Administration

MSG was administrated intravenously (iv) to healthy intact rats through the dorsal lateral vein. Dosage administration of MSG was 10 mmol/L (0.1 ml) [41]. All drugs were purchased from Sigma-Aldrich Co®.

2.3 Blood Samples

Prior to blood collection, rats were anesthetized with an intraperitoneal injection of chloral hydrate. Blood was obtained via cardiac puncture using a No 23 gauge needle attached to a plastic disposable syringe and placed into different tubes. Blood samples for blood analysis were placed into EDTA bottles, whereas blood for aggregation studies was anticoagulated with 3.8% trisodium citrate in a ratio of 9 parts of blood to 1 part of anticoagulant [42]. Differential centrifugation was used in order to obtain platelet rich (PRP) and platelet poor plasma (PPP) [43].

2.3.1 Complete blood count (CBC)

An analyzer ADVIA 120 Hematology System was used in order to perform CBC. The following parameters were measured or analyzed: White Blood Cells (WBC), Red Blood Cells (RBC), Hemoglobin (Hb), Hematocrit (Hct), Mean Cell Volume (MCV), Mean Cell Hemoglobin (MCH), Mean Cell Hemoglobin Concentration (MCHC), Platelets (PLT) and differential results (neutrophils, lymphocytes, monocytes, eosinophils, basophils) in absolute numbers.

2.3.2 Aggregation studies

Platelet aggregation studies were performed with a PAP4 Platelet aggregation profiler equipped with a recorder. 0.05 ml of an antiaggregating agent was added to an aliquot of 0.045 ml of PRP equilibrated to 37°C and each aggregation was recorded for 5 minutes. Aggregations were quantified as the maximum extent (intensity) of light transmittance in stimulated PRP. Aggregations were induced with adenosine diphosphate (ADP) and collagen [44].

2.3.3 Bone marrow smears

Bone marrow was collected from the femur rats, were put to death and then the bone marrow

cavity was exposed after incision of the bone. The whole procedure did not last more than 3 minutes so as to avoid cell damage [45]. Up to 500 cells were studied for each smear by two individual investigators and results were compared.

Complete blood counts, aggregation studies and bone marrow smear examination were performed 12 (MSG-12h group) and 24 (MSG-24h group) hours after drug administration and the results were compared to a control group free of drug administration.

2.4 Statistical Analysis

Blood count values, platelet aggregation were normally distributed and analyzed with unpaired t-test. For all tests, the level of significance was set at 5% ($p<0.05$). Results are expressed as mean±SEM.

3. RESULTS

3.1 CBC

When comparing MSG-12h group CBC results to control group statistical important differences were identified in 7 different parameters.

To be more specific, there was an increase in Hct value ranging from 42.03±1.6 in control group to 44.6±0.76 in MSG-12h (Fig. 1a) and an increase in MCH value ranging from 17.56±0.38 in control group to 18.26±0.23 in MSG-12h (Fig. 1b). Hb levels were also statistically important increased from 14.33±0.46 to 15.64±0.47 in control and MSG-12h group respectively (Fig. 1c).

Statistical important differences were also identified in 7 parameters when comparing MSG-24h group CBC results to control group of which 5 different parameters were also observed when comparing MSG-12h CBC results to control group.

Hct and Hb values remained statistically important increased compared to control group (44.24±1.22 and 15.66±0.29 respectively) (Fig. 1a and 1c).

White Blood Count was negatively affected with a statistical important decrease in terms of absolute neutrophil count (77.6±15.31 in MSG-12h group vs 438.66±108 in control group), absolute lymphocyte count (267±34.17 in MSG-12h group vs 509±134 in control group), absolute monocyte count (11.8±1.88 in MSG-12h group vs 50±9.5 in control group) and absolute eosinophil count (6.2±1.28 in MSG-12h group vs 27.66±3.84 in control group) (Fig. 2).

As far as absolute lymphocyte, monocyte and eosinophil counts are concerned, when comparing MSG-24h group CBC results to control group, they were statistically important decreased compared to control group, 213±57.88, 18.4±2.46 and 4.2±0.96 respectively (Fig. 2).

3.2 Aggregation Studies

A statistical important transient increase in ADP induced aggregation curve was identified in MSG-12h group that went back to normal levels 12 hours later (MSG-24h group) (Fig. 3). Collagen induced aggregation was not affected in any group.

3.3 Bone Marrow Studies

Even though bone marrow cytology did not seem to be affected in a statistically important way in MSG-12h group, this did not seem to be the case for MSG-24h group.

In more detail, in MSG-24h group, cellularity was affected both in white and erythroid blood cell lineage even though total red/white blood cell precursors ratio was not affected .

To be more specific, pronormoblast cell count was decreased from 4.3±0.36 in control group to 2.19±0.18 in MSG-24h group, and polychromatic normoblast cell count was reduced from 13.22±1 to 6±0.42. Band neutrophil cell count was increased from 4.6±0.49 in control group to 7.26±0.75 in MSG-24h group and eosinophil count was increased from 3.26±0.4 to 5.99±0.47 (Fig. 4).

Also megakaryocytes were found to be normal both in morphology and in cellularity.

4. DISCUSSION

Lymphocyte, monocyte and eosinophil counts showed a statistically significant decrease 12 and 24 hours after MSG infusion, whereas neutrophil count was only affected 12 hours after MSG infusion and went back to normal 12 hours later. All of the above indicate that white blood cells are affected by glutamate acid. Glutamine and/or

glutamate in a certain concentration may act as modulators of lymphocytes cell cycle [46]. Glutamine is used for neutrophil and monocyte metabolism [47] in exchange for glutamate release [48,49]. A possible explanation concerning the mechanism through which MSG administration affects cells is that glutamate receptor antagonists act upon channels that release glutamate acid so that a potential extracellular increase of the later consequently leads to a transient pause of those channels and thus, to an increase in intracellular glutamate levels. The later translating to cell cycle deregulation and even cell death in analogy to NR1 activation in lymphocytes [37] but does that mean that all WBC express glutamate receptors? We already know that the administration of 0.5 mM glutamate can induce a decrease in glutamine use and that glutamine is widely used by lymphocytes, macrophages and neutrophils in a way that it affects many of their natural functions (T-cell proliferation, phagocytocis, antigen presentation and apoptosis) [48,50,51]. Glutamate is also important for NADPH production [52]. As far as eosinophil reduction is concerned, we can only make analogous speculations. Moreover, based on observation Weu et al. [53] that both NMDA and non-NMDA antagonists restrain granulocyte progenitor cells after seizures in adult rats in the brain, our results suggest a similar affect in bone marrow progenitors.

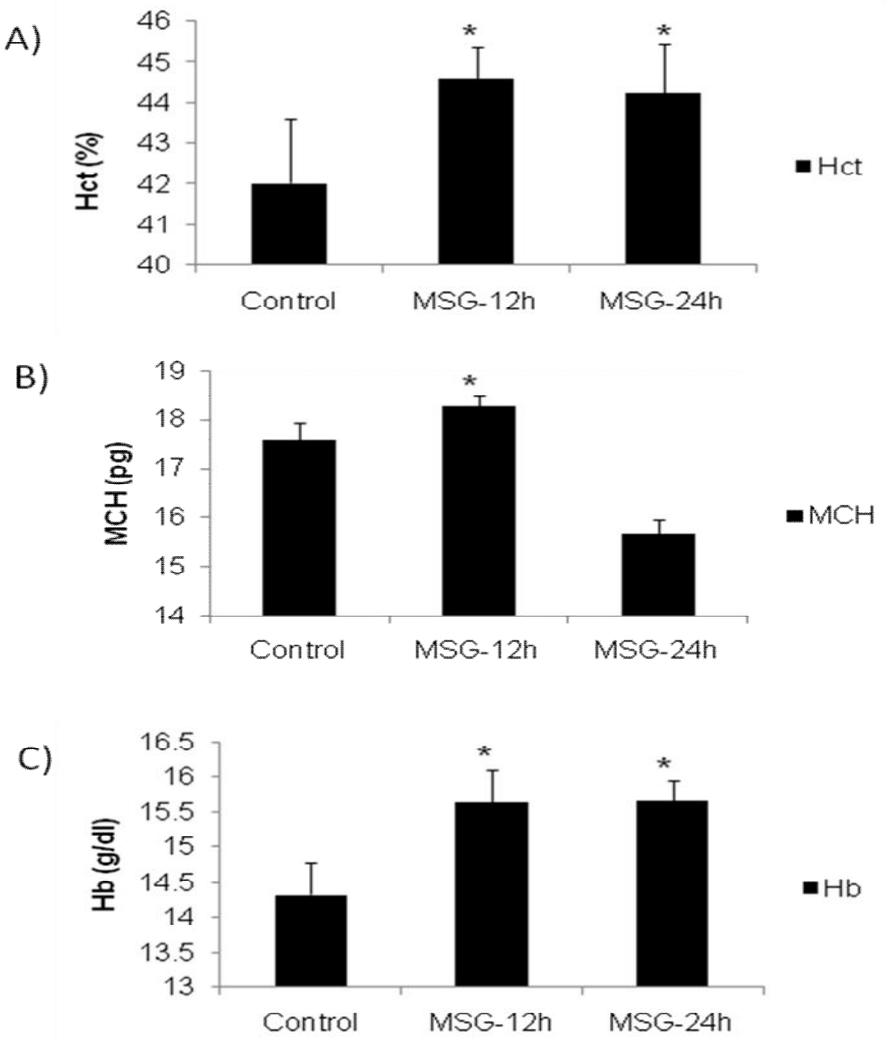

Fig. 1. Statistical important differences in Hb, Hct and MCH values compared to control group were noticed both in MSG-12h and MSG-24h group

*Significant from normal control, * P < 0.05*

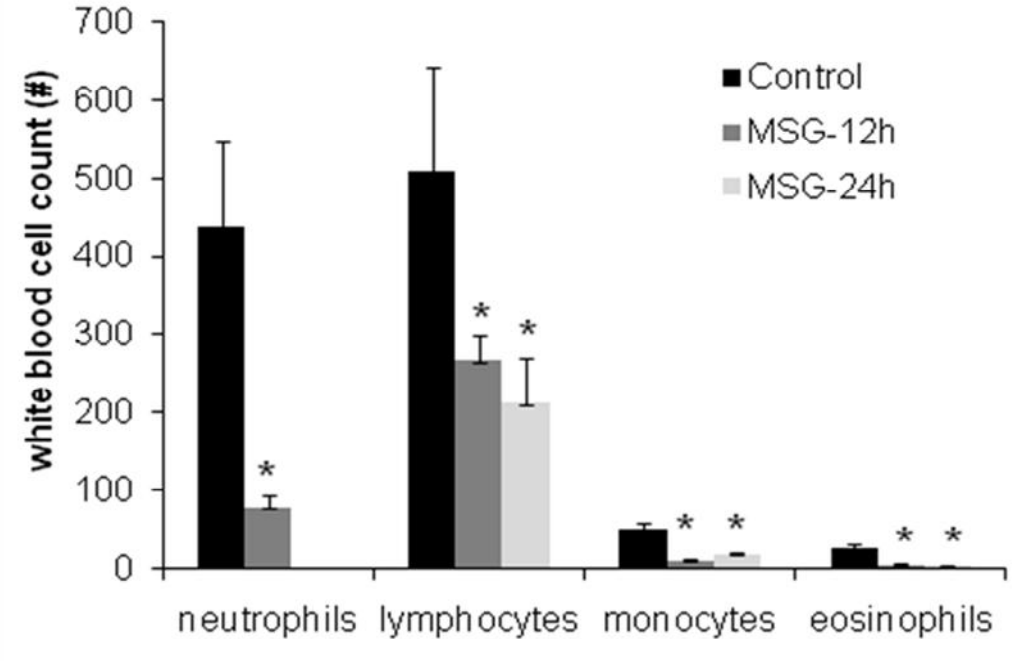

Fig. 2. Neutrophil, lymphocyte, monocyte and eosinophil white blood cell count was affected both in MSG-12h and MSG-24h groups

*Significant from normal control, * P < 0.05*

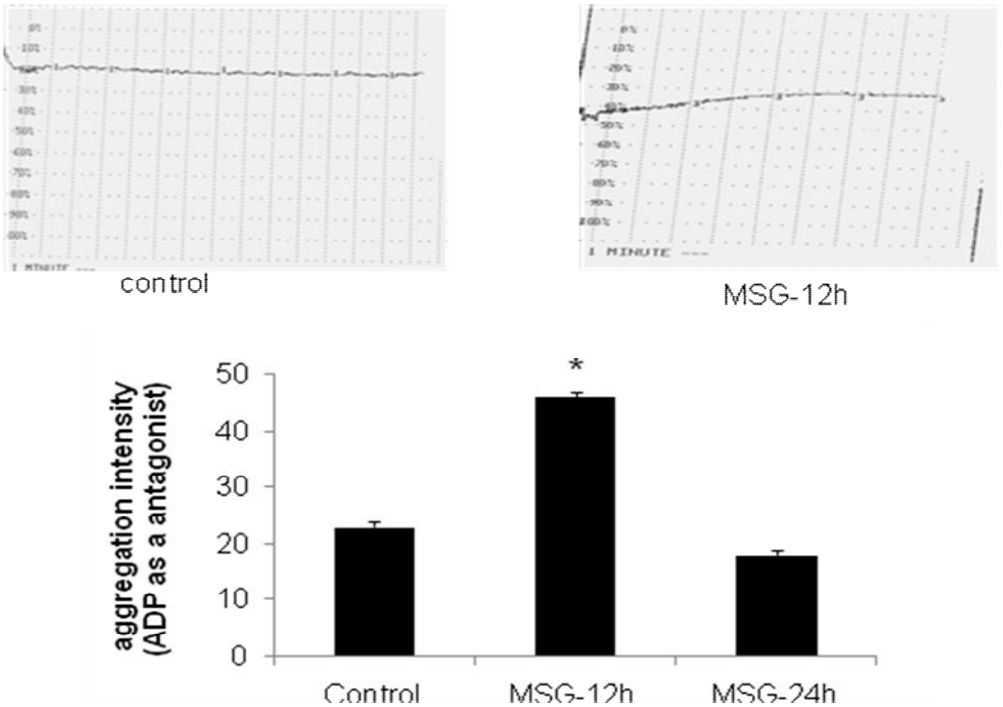

Fig. 3. Comparison of the aggregation intensity that reached a statistically significant level between MSG and control group with ADP as an antagonist

*Significant from normal control, * P < 0.05*

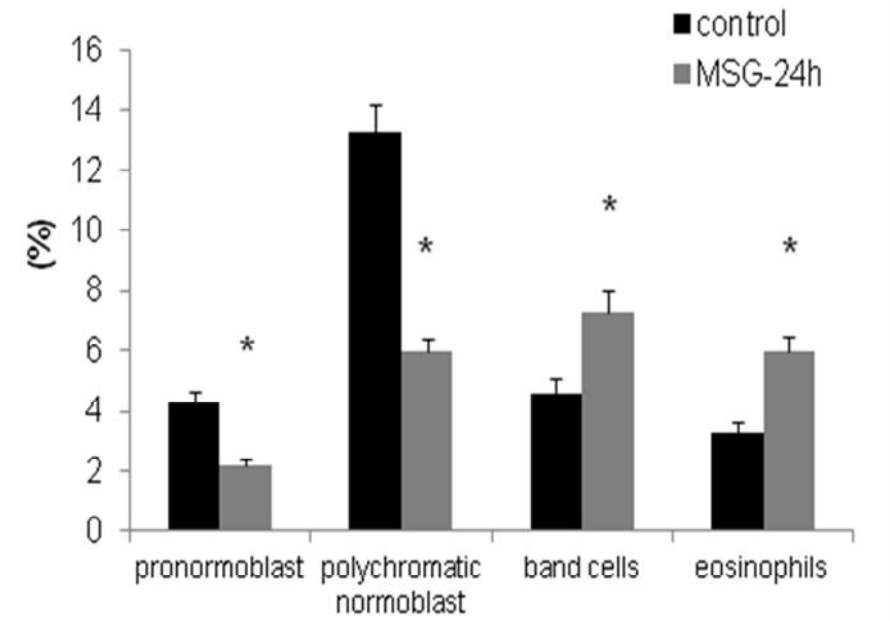

Fig. 4. Statistical important differences in bone marrow counts were noticed in MSG-24h group
*Significant from normal control, *P<0.05*

Turning to erythroid series, we know that even though red blood cell glutamate concentration is twice as much as plasma's [54] red blood cells and their progenitors, are incapable of either release or intake [55]. Divino et al. [56] have shown that intracellular glutamate is affected by insulin levels and that insulin growth factor can drive glutamate in and out of the cells. Also, subcutaneous administration of MSG has been shown to induce oxidative stress in adult male rats [57].

Last but not least, Hct and Hb were found to be increased both 12 and 24 hours after MSG administration, whereas MCH values were found to be increased only 12 hours after and MCHC values 24 hours after the administration. Intravenous administration of glutamate ionotropic antagonist MK801 (1 mg/kg) in cats has been shown to induce apnea [58] while reducing phrenic neurogram (PN) and inspiratory-synchronous (ISSN) by 38% and 84%, respectively, whereas the administration of another antagonist, namely NBQX, negatively affected PN and ISSN 54% and 60% respectively. As a result, less oxygen would be provided in tissues and more hemoglobin production.

Pierrefiche et al. [59] also showed that both ionotropic antagonists, especially non-NMDA antagonists, may have a negative effect in respiratory system function. Even though we cannot directly explain Hct and Hb values increase after MSG administrations speculations can be made with regards to a defect in respiratory system function since we already know that the activation of glutamate receptors in lungs and airways can be a factor for asthma pathogenesis [60,61].

MSG administration affected platelet aggregation in a statistical important way but this effect lasted only 12 hours and turned back to normal 24 after the administration. Platelets express AMPAR subunits Glu R1-4 and GluR1 subunit is expressed on their surface [62]. They are also capable of releasing and saving glutamate through a transport system [34].

Glutamate is known to increase platelet aggregation to Platelet G-protein coupled receptors-GPCR like ADP, whereas it has no affect in non-GPCR receptors like collagen [34]. These results are in accordance with our study but are opposed to an older one that underlies that glutamate has a negative effect in platelet aggregation [63]. The best explanation for this contradiction is that glutamate effects differ in different concentrations as has already been suggested for lymphocytes [46].

Pronormoblast and polychromatic normoblast count was statistically important decreased 24

hours after MSG administration whereas band cells and eosinophils were statistically important increased. At the same time, in peripheral blood, Hct and Hb values were statistically important increased and eosinophil count was statistically important decreased. In addition, 12 hours after MSG administration, Hct, MCH and Hb values were increased and neutrophil and lymphocyte counts were reduced. Based on the above we can speculate the following: Pronormoblast and polychromatic normoblast decrease may come as a result to the increase noted in peripheral blood. The same goes to the decrease in neutrophil cells and eosinophil progenitor cells.

5. CONCLUSION

The intravenous administration of glutamate agonist MSG showed to have an effect on the hematopoietic system of healthy intact Wistar rats that varied at different time points.

We show for the first time an in vivo effect of glutamate in the bone marrow of Wistar rats. Our studies strongly depend on cytomorphology and further investigation is needed with immunohistochemistry and/or flow cytometry tests.

In addition, a longer follow-up upon drug administration is needed in order to identify possible long time effects.

CONSENT

It is not applicable.

ETHICAL APPROVAL

All authors hereby declare that experiments have been conducted in accordance with the European Communities Council Directive of 24 November 1986 (86/609/EEC) and the “Principles of laboratory animal care” (NIH publication No. 85-23, revised 1985) were followed, as well as specific national laws where applicable. All experiments were approved by the Ethical Committee of the School of Medicine of Aristotle University of Thessaloniki.

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COMPETING INTERESTS

The authors declared no conflict of interest.

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