

Investigation of Anti-Inflammatory Activity of Hydroxyzine Hydrochloride in Macrophage Cells

Hidroksizin Hidroklorürün Makrofaj Hücrelerinde Anti-inflamatuar Aktivitesinin Araştırılması

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Öz

Amaç	Depresyon, hastaların sağlıkla ilgili yaşam kalitesini etkileyen yaygın bir ruhsal bozukluktur. Antihistaminikler arasında yer alan hidroksizin hidroklorür (HHCL) genellikle alerjik tedavilerde kullanılmaktadır. Ayrıca HHCL, son yıllarda anksiyete, zihinsel sıkıntı ve stres tedavisinde rutin olarak kullanılmaktadır. Hidroksizin klorür ile ilgili birçok çalışma olmasına rağmen literatürde bu ilacın bağışıklık sistemi üzerindeki etkileri hakkında yeterli bilgi bulunmamaktadır.
Gereç ve Yöntemler	Önceki çalışmalar, bazı antidepresanların anti-inflamatuar ve anti-analjezik olduğunu düşündürmektedir. Bu nedenle, bu ilaçlardan birinin, Hidroksizin hidroklorürün memeli makrofajları üzerindeki immünomodülatör aktiviteleri, fosforile edilmiş (aktif) p38 MAPK ve PI3K proteinlerinin olası hücre içi etki mekanizmalarını belirlemek için akış sitometrisinde analiz edildi.
Bulgular	Makrofaj hücrelerine uygulanan Hidroksizin hidroklorürün, p38 MAPK ve PI3K proteinlerinin düzeylerinde önemli bir azalmaya neden olduğu bulunmuştur.
Sonuç	Bu verilere göre Hidroksizin hidroklorür (HHCL), makrofajlar üzerinde farklı etkilere sahipti. HHCL, aktive olmayan makrofajlarda hafif bir inflammatuar yanıtı tetiklerken; zaten aktive olan makrofajlar üzerinde anti-inflamatuar etkileri vardı. HHCL'nin hücre içi etki mekanizmaları, kısmen p38 MAPK ve PI3K yolları aracılığıyla olmuştur.
Anahtar kelimeler	Antiinflatuar aktivite; hidroksizin hidroklorür, makrofajlar; enflamasyon; depresyon.

Abstract

Objective	Depression is a common mental disorder that affects patients' health-related quality of life. Hydroxyzine hydrochloride (HHCL), which is among the antihistamines, is generally used in allergic treatments. Also, HHCL, has been routinely used in the treatment of anxiety, mental distress and stress in recent years. Although there are many studies on hydroxyzine chloride, there is not enough information in the literature about the effects of this drug on the immune system.
Materials and Methods	Previous studies suggest that some antidepressants are anti-inflammatory and anti-analgesic. Therefore, the immunomodulatory activities of one of these drugs, Hydroxyzine hydrochloride, on mammalian macrophages were analyzed in flow cytometry to determine the possible intracellular mechanisms of action of the phosphorylated (active) p38 MAPK and PI3K proteins.
Results	It has been found that Hydroxyzine hydrochloride applied to macrophage cells causes a significant decrease in the levels of p38 MAPK and PI3K proteins.
Conclusion	According to these data, Hydroxyzine hydrochloride (HHCL), had different effects on macrophages. While HHCL triggers a mild inflammatory response in non-activated macrophages; it had anti-inflammatory effects on already activated macrophages. Intracellular mechanisms of action of HHCL were partially via the p38 MAPK and PI3K pathways.
Keywords	Anti-inflammatory activity; hydroxyzine hydrochloride, macrophages; inflammation; depression

INTRODUCTION

Depression is one of the main causes of disability, accounting for a significant percentage of morbidity worldwide. Depression and its symptoms are spreading around the world lately. Depression is one of the disorders that significantly affects the quality of life.^{1,2} Around the world, 25% of women and 12% of men show signs of depression. In particular, the rate of depression and its symptoms in sick individuals is higher than in healthy individuals.^{2,3} There is an important relationship between anxiety and depression.^{4,5} Anxiety disorders are usually chronic and have a high probability of recurrence. They are associated with psychological disturbances, difficulties in social and professional affairs, poor life quality, and overall significant economic distress.⁶ Anxiety disorders in humans range from 2.4% to 29.8%.⁷ Antidepressant drugs such as duloxetine, a strong serotonin (5-HT) and norepinephrine (NE) reuptake inhibitor and a weak dopamine (DA) reuptake inhibitor, are used in the treatment of anxiety disorders.⁸ Hydroxyzine, one of these antidepressant drugs, is an antagonist of histamine receptors.⁹

Antihistamines are drugs commonly used for patients seeking primary care and over-the-counter treatment. Hydroxyzine hydrochloride (HHCL), one of the antihistamines, is a member of the H1 receptor antagonists used in histamine-mediated pruritus in allergic conditions such as atopic or contact dermatoses. This drug has anxiolytic, mild anti-obsessive and antipsychotic effects and antispasmodic, sedative, antiemetic and anticholinergic properties due to its antagonistic effects on various receptor systems in the brain.^{10,11} Also, this drug has antispasmodic, sedative, antiemetic and anticholinergic properties. In recent years, the use of antidepressant drugs has been increasing intensively around the world. There are many clinical studies show that significant regression in disease symptoms when these drugs are used for psychological disorders. In 2020 World Health Organization data; about 264 million people of all ages worldwide use antidepressants for depression.¹² In our study, we tested the possible effects

of Hydroxyzine Hydrochloride on the immune system on non-activated mammalian macrophages. This drug was also tested for its immunomodulatory activities on LPS-activated macrophages. The intracellular mechanisms of action of macrophages treated with HHCL were studied by measuring the active phosphorylated levels of p38 MAPK and PI3K. These pathways are essential for inflammatory functions.¹³⁻¹⁵

MATERIAL and METHODS

After J774.2 mammalian macrophage cells purchased from ATCC reached sufficient numbers for the experiment, the cell concentration per well was seeded at 106 cells/well in 1 mL and then cells were incubated overnight in a humidified incubator at 37 °C with 5% CO₂. Next, hydroxyzine hydrochloride (HHCL) (Sigma Aldrich) was resuspended in 1 mL of sterile tissue culture grade DMSO at a concentration of 20 µg/mL Hydroxyzine hydrochloride (HHCL), 1 µg/mL LPS (lipopolysaccharide). was added to each suitable well, with or without LPS. The samples were kept in a humid incubator for 24 hours. There was no compound or LPS in the control group, 10µg/mL Salicylic acid (SA) and 1µg/mL LPS were added to the negative control group, 1µg/mL LPS to the positive control group, 1µg/mL LPS to another positive control group. 5µg/mL Al₂O₃ and mL LPS were added to the appropriate wells. After a 24-hour incubation period, supernatants were collected to analyze intracellular levels of phosphorylated p38 and phosphorylated PI3K. Cell fixation (BD Fixation Buffer I (557870)) and Cell permeabilization (D PERM Buffer III (558050)) were done with followed by intracellular staining with BD PE Mouse anti-PI3K (Invitrogen) and BD PE Mouse Anti-p38 MAPK. (pT180/pY182) according to protocol BD protocol. Analysis was performed by BD FACS ARIA III for Ayaz et al, 2021¹⁶, Yüzer et al., 2019¹⁷.

Statistical Analysis

Statistical analysis was performed using GraphPad Prism Software version 5. Unpaired two-tailed t-test was used to

reveal statistical significance.

RESULTS

The Hydroxyzine HHCL anti-depressant was partially responsible for the anti-inflammatory effect through the PI3K and p38 pathways.

Many factors such as p38 mitogen-activated protein kinase (MAPK) and phosphoinositide 3-kinase/Akt (PI3K/Akt) pathways have critical role in inflammation, stress, apoptosis, cell cycle and cell growth. In this study, the ef-

fect of HHCL on p38 and PI3K pathways in macrophage cells were studied. J774.2 macrophage cells were treated with LPS, LPS+SA, LPS+AL203, 20µg/mL HHCL and LPS+20µg/mL HHCL for 24 hours. The percentages of PI3K and P38 positive cells increased in the presence of the drug molecule without LPS (Table1, Figure 1 and Figure 2), but this increase was statistically significant only in PI3K positive cells. These results suggest that Hydroxyzine HCL (HHCL) plays an important role as an immunostimulator via the PI3K pathway. (Table 1 and Figure 1)

Variables	Control	LPS	LPS SA	LPS Al ₂ O ₃	20 µg/ml of HHCL	LPS 20µg/ml + HHCL
PI3K-	87,33±2,01***	52,70±2,93***	50,13±8,90	42,43±2,48	35,06±5,55***	61,20±1,41***
PI3K+	12,03±1,83	46,80±2,72	49,33±8,91	57,16±2,34	64,33±5,29***	38,53±1,40***
P38-	77,00±8,07	62,56±6,20	56,83±11,80	57,63±3,91	61,50±4,00	87,26±3,86*
P38+	23,40±8,15	37,80±6,06	43,76±11,90	42,90±3,86	39,06±4,11	23,30±14,5*

Statistical significance values: When compared to the Control: *P≤0.05, **P≤0.005, ***P≤0.001. SD: Standard Deviation

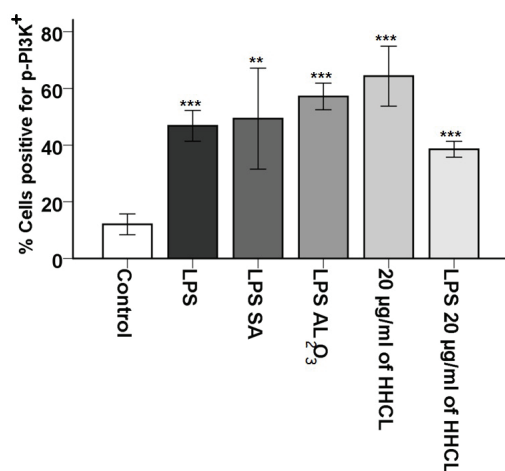


Figure 1. 1×10^6 cells/mL J774.2 macrophage cell concentration Percentage of PI3K positive cells after stimulation with LPS, LPS and Salicylic acid, LPS and Al₂O₃, 20µg/mL HHCL and LPS+20µg/mL and HHCL for 24 hours. Negative control; DMSO, positive control; 1 µg/mL LPS and DMSO were used. Not stained control was used as the baseline evaluation standard 1 in the ground induction calculations. Statistical analyzes were performed using Student's t test $p < 0.0001$ $N = 3$.

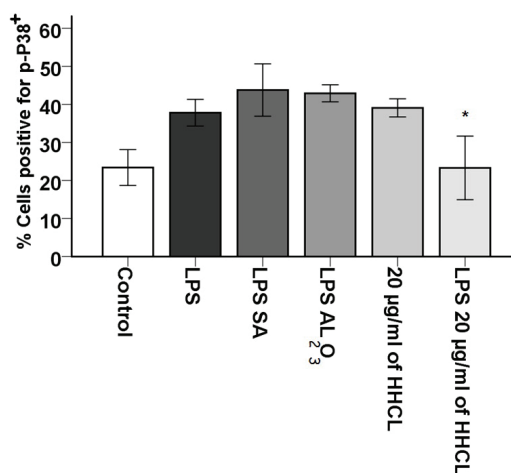


Figure 2. 1×10^6 cells/mL J774.2 macrophage cell concentration Percentage of P38 positive cells after stimulation with LPS, LPS and Salicylic acid, LPS and Al₂O₃, 20µg/mL HHCL and LPS+20µg/mL and HHCL for 24 hours. Negative control; DMSO, positive control; 1 µg/mL LPS and DMSO were used. Not stained control was used as the baseline evaluation standard 1 in the ground induction calculations. Statistical analyzes were performed using Student's t test $p < 0.0001$ $N = 3$.

DISCUSSION

Chronic itching is a common condition that affects patients' quality of life and also causes serious psychological disorders.¹⁸⁻²⁰ Although there are various treatment methods available today, treatment is more difficult if itching cannot be associated with a skin disease.²¹ Antihistamines are used as standard treatment in these diseases because they are effective in the treatment of chronic itching. In some guidelines, second-generation agents are also recommended as first-line agents. However, large variations can occur in responses to second-generation agents. Therefore, first-generation antihistamines are recommended for patients who do not gain adequate benefit despite increased doses of second-generation agents. However, previous studies have reported less or less symptomatic sedative and motor impairment potentials with second-generation agents.¹⁸⁻²² The European Chronic Itch Guidelines recommend that hydroxyzine hydrochloride be used as the first-line treatment for pruritus owing to its anti-itching, anti-anxiety and soothing properties.^{20,23} Based on in-vivo studies, hydroxyzine hydrochloride has been reported to be the most potent antihistamine for the treatment of chronic pruritus.²³ Non-sedating second-generation antihistamines have anti-inflammatory activity and their effects last longer, however first-generation antihistamines 18 are preferred for relief from chronic itching as they are considered less effective than sedatives. In addition, increasing the dose of non-sedating second-generation agents may also cause sedative side effects.²⁴

In this study, cells were measured for phosphorylated p38 and PI3K levels to determine the intracellular mechanism of action of hydroxyzine hydrochloride. We found that the concentration of 20 µg/mL at which hydroxyzine hydrochloride showed the highest activity increased the percentage of p38 and PI3K positive cells, similar to cells treated with LPS. Based on these results, we show that hydroxyzine hydrochloride acts through these pathways to exert its immunostimulatory activities. Schindler et al. indicate that the p38 pathway is targeted in drugs used in the

treatment of inflammatory diseases associated with high cytokine production.²⁵ In previous studies, it has been shown that the p38 pathway plays a role in the production of TNF-α and IL-6 by LPS-induced macrophages.^{26,27} Cho et al. showed that paroxetine induced apoptosis in MCF 7 breast cancer cells according as the Ca+2 and p38 pathway.²⁸

PI3K is known as an effective intracellular pathway in regulating cell metabolism, proliferation and apoptosis. Mutations in the PI3K pathway have been seen in many human cancers. Phospholipid secondary messengers produced by PI3Ks provide a common mechanism for cell fate in intracellular signal transduction.^{29,30} The increase in cytokine levels such as interleukins, TNF-α and IFN-γ, which play a role in the treatment of depression with some antidepressants, contribute to cell-mediated immune activation. Our results show that HHCL induces inflammatory responses by macrophages via the PI3K pathway.

Our work on macrophages suggests that it should be used with caution in patients suffering from inflammatory disorders associated with depression, as HHCL will further increase inflammation through macrophages. Our findings show that this drug molecule induces the inflammatory activity of macrophages by activating the p38 and PI3K pathways. In our future work, we will focus specifically on the inflammatory reaction of HHCL and other antidepressants in different immune system cells in order to fully explain their possible effects on overall immune system activity during treatment.

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Human and Animal Ethics

Not applicable.

Conflict of Interest

Author do not have any financial or non-financial conflict

of interest to declare.

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