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Effect of phenelzine and serotonin on RAW264.7 macrophage cell viability

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Abstract

Background: Serotonin is a neurotransmitter with extensive physiological effects on the Central Nervous System (CNS) and various biological functions, including the regulation of immunity through 5-hydroxytryptamine receptors (5-HTRs) expressed by immune cells such as macrophages. Phenelzine, a medication used in managing treatment-resistant depression, acts as a potent monoamine oxidase inhibitor (MAOI). This enzyme metabolizes serotonin into 5-hydroxyindoleacetic acid (5-HIAA). Antidepressants e.g., Phenelzine may benefit patients with neurological disorders, who can also be prone to immune-related conditions and cancer. This study aimed to investigate the cytotoxic effects of Phenelzine, serotonin, and 5-HIAA on RAW264.7 macrophages.

Methods: We cultured RAW264.7 macrophages as a model that could express transporter receptors and enzymes associated with serotonin. We utilized MTT assay to evaluate the survival of RAW264.7 cells exposed to different concentrations of Phenelzine, serotonin, and 5-HIAA, pre-treated with lipopolysaccharide (LPS).

Results: Our findings revealed that LPS-treated RAW264.7 cells exhibited increased resistance to the cytotoxic effects of Phenelzine. Treatment with serotonin resulted in a concentration-dependent increase in RAW264.7 cell proliferation. In contrast, 5-HIAA did not significantly impact cell viability.

Conclusion: The present study reveals the effect of Phenelzine and serotonin on viability of RAW264.7 macrophages, particularly in the context of inflammation. It demonstrates increased resistance to the cytotoxic effects of Phenelzine in RAW264.7 cells treated with LPS. Our study contributes to a broader understanding of the potential systemic impacts of antidepressant medications and the intricate interplay between the serotonergic system and immune responses.

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Highlights

What is current knowledge?

- Phenelzine, a serotonin-modulating drug, is known for its role in treating neurological disorders and may also influence immune response.
- Macrophages are key elements of the innate immunity and express serotonin receptors that can impact their viability and functionality.
- Data suggests notable bidirectional interplay between certain inflammation-related diseases and depression, considering the dual role of serotonin in the CNS and immune system regulation.

What is new here?

- Our study reveals that Phenelzine affects the viability of macrophages, which can have implications for the immune response in patients.
- The presence of LPS diminishes the cytotoxic effects of Phenelzine on RAW264.7 macrophages, suggesting a complex interaction with inflammatory conditions.

Introduction

Serotonin (5-HT) is a monoamine neurotransmitter that plays a significant role in regulating various behavioral and biological functions in the body. It is involved in psychological processes in the Central Nervous System (CNS) and also in peripheral tissues such as bone and intestine (1-3). While serotonin is commonly known as a neurotransmitter in the brain, around 95% of this molecule in the body is actually produced, stored, and released by enterochromaffin (EC) cells in the intestinal mucosa and the tryptophan hydroxylase 1 (TPH 1) enzyme (4-6). The intestine contains various types of cells, including a significant number of peripheral immune cells that help regulate the immune systems in the gastrointestinal tract (GIT) (7).

Macrophages are key elements of the innate immunity that are present throughout various tissues, including GIT and are crucial for maintaining homeostasis. They also express serotonin receptors, including 5-HT1A, 1B, 1E, 2A, 2B, 2C, 3, 4, and 7, as well as tryptophan hydroxylase (TPH), monoamine oxidase (MAO), and serotonin transporter (SERT). Several studies have confirmed the expression of serotonin receptors, particularly 1B and 2B, as well as enzymes like TPH, MAO, and SERT transporter in RAW264.7 cells (8-13).

A wide range of serotonin-modulating medications have been developed for the treatment of neurological disorders. These medications have also shown effectiveness in improving symptoms of certain autoimmune conditions and cancers (7,14). Monoamine oxidase inhibitors (MAOIs) were initially introduced in the 1950s as a type of antidepressant medication and have since been used to treat various forms of depression and other nervous system disorders like panic disorder, social phobia, and atypical depression (15-17).

Monoamine oxidase (MAO) has two isoforms (MAO-A and MAO-B) and breaks down serotonin into 5-hydroxyindole acetic acid (5-HIAA) through oxidative deamination (18). While monoamine oxidase A (MAO-A) is mainly associated with its role in the nervous system, several studies have identified MAO-A as a marker of anti-inflammatory phenotype activation in monocyte/macrophage cells (19). Phenelzine (Nardil) is a non-selective and irreversible inhibitor of the MAO enzyme. This means it inhibits both isoforms, MAO-A and MAO-B, and covalently binds to the enzyme without detaching from the binding site (20-22).

Data suggests that reduced serotonergic activity may compromise the mechanisms involved in maintaining recovery from depression (23-25). Lower levels of tryptophan in the plasma, the precursor amino acid of serotonin, are a significant finding in patients with more severe forms of depression (26). Furthermore, inflammation can induce depression in susceptible individuals by reducing plasma tryptophan and decreasing brain serotonergic activity which may disrupt mechanisms related to maintaining recovery from depression, rather than having an independent and primary effect in all vulnerable individuals (27,28).

Considering the notable bidirectional interplay between certain inflammation-related diseases and depression (Figure 1), and acknowledging that antidepressants like Phenelzine, prescribed for individuals with neurological disorders who may concurrently be susceptible to immune-related conditions and cancer, may influence inflammatory conditions and the viability of macrophages through different mechanisms. RAW264.7 macrophages are essential in modulating inflammatory conditions and are able to express receptors, transporters, and enzymes associated with serotonin. Therefore, this study aimed to investigate the effect of Phenelzine, serotonin, and 5-HIAA on the viability of RAW264.7 macrophages, particularly in inflammatory conditions induced by lipopolysaccharide (LPS).

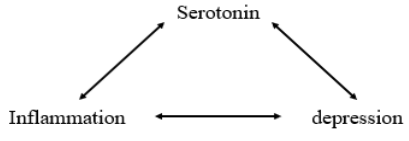

Figure 1. Schematic diagram of the relationship between serotonergic system, inflammation, and depression.

Methods

Cell culture

The RAW264.7 cell line was cultured in RPMI medium supplemented with 10% fetal bovine serum (FBS) at 37 °C in a 5% CO2 environment. These cell lines were obtained as flask cultures from the Cell Bank of the Pasteur Institute of Iran. All culturing steps were performed under sterile conditions. A cell suspension containing 20 × 103 cells in a volume of 200 µL was added to each well of a 96-well plate. After 24 hours, LPS was added at a concentration of 100 nM per well. Subsequently, different concentrations of Phenelzine, 5-HIAA, and Serotonin were added to the wells to achieve the desired concentrations. RAW264.7 cells were incubated for 24 hours before conducting the MTT assay.

MTT assay

A 5 mg/ml MTT (3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide) solution was prepared, filtered, and covered with foil. The second column of the plate was designated as the control. The liquid above the cell monolayer (Supernatant) was aspirated, and each well received 100 µL of culture medium and 20 µL of MTT solution. After further incubation, the formazan crystals were solubilized by adding 100 µL of dimethyl sulfoxide (DMSO). The absorbance was read using an ELISA Reader at 630 nm wavelength with a reference at 570 nm. Cell viability was calculated by the following formula:

The vitality percentage of cells =100×(a/b)
a = Optical Density (OD) of the test sample minus the blank's OD
b = OD of the control minus the blank's OD

Data analysis

The results, derived from three replicates, were presented as mean ± standard deviation (SD). All calculations were performed using Microsoft Excel software (Microsoft, Redmond, WA, USA). Statistical analysis was carried out using the independent sample Kruskal-Wallis test with the assistance of SPSS software (IBM Inc., Armonk, NY, USA). The significance levels of the data are denoted as follows: *P <0.05*, **P <0.01**, and ***P <0.001***.

Results

Effect of DMSO and LPS on RAW264.7 cell viability

Treatment of RAW264.7 cells with 0.3% DMSO, equivalent to the highest concentration used as a solvent for 5-HIAA, and with 100 ng/ml LPS for 24 hours did not result in any significant cytotoxic effects (P-value =0.124, P-value =0.466, respectively) (Figure 2).

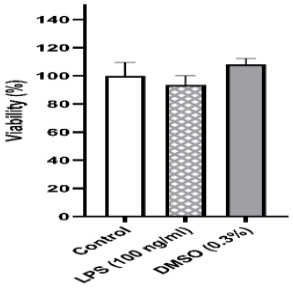

Figure 2. Viability of RAW264.7 cells after 24 hours of treatment with 100 ng/ml LPS and 0.3% DMSO showed no significant changes. The mean viability ± SD was calculated using the formula (OD test/OD control × 100) and analyzed using the Kruskal-Wallis test (* p <0.05, ** p <0.01), *** p <0.001).

Cytotoxic effect of Serotonin on RAW264.7 cells

RAW264.7 cells treated with 10 µM and 1 mM concentrations of serotonin for 24 hours increased the viability of RAW264.7 cells by 15% (P-value =0.018) and 35% (P-value <0.000) respectively, while treatment with 0.1 µM serotonin did not result in a statistically significant impact on the cell viability (P-value = 1) (Figure 3).

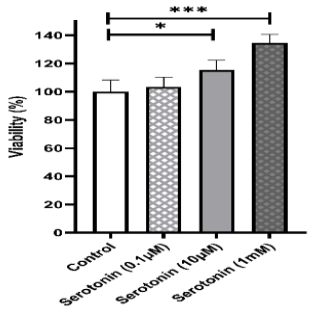

Figure 3. Viability of RAW264.7 cells after 24 hours with 0.1 µM serotonin showed no significant change, whereas concentrations of 10 µM and 1 mM serotonin increased the viability of RAW264.7 cells. All groups were pre-treated with LPS (100 ng/ml). The mean viability ± SD was calculated using the formula (OD test/OD control × 100) and analyzed using the Kruskal-Wallis test (* p <0.05, ** p <0.01), *** p < 0.001).

Cytotoxic effect of 5-HIAA on RAW264.7 cells

Treatment of RAW264.7 cells with three different concentrations of 5-HIAA (1 µM, 10 µM, and 100 µM) for 24 hours did not induce any statistically significant changes in the cell viability (P-value = 0.27, P-value = 1, P-value = 0.218, respectively) (Figure 4).

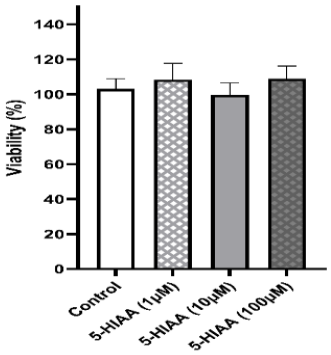

Figure 4. Viability of RAW264.7 cells after 24 hours of treatment with 1 µM, 10 µM, and 100 µM 5-HIAA showed no significant changes. All groups were pre-treated with LPS (100 ng/ml). The mean viability ± SD was calculated using the formula (OD test/OD control × 100) and analyzed using the Kruskal-Wallis test (* p <0.05, ** p <0.01), *** p <0.001).

Cytotoxic effect of Phenelzine on RAW264.7 cells

Initially, RAW264.7 cells treated with 10 µM, 20 µM, and 40 µM concentrations of Phenelzine for 24 hours without prior LPS treatment showed a significant cytotoxic effect, reducing viability to approximately 10% (P-value <0.000) (Figure 5). Subsequent treatment of RAW264.7 cells with 1 µM Phenelzine for 24 hours following pre-treatment with LPS (100 ng/ml) did not cause a statistically significant change in viability of these cells (P-value = 1); however,10 µM phenelzine reduced viability by approximately 15%, which was not statistically significant (P-value = 0.119). Furthermore, 20 µM and 40 µM Phenelzine treatments resulted in a significant cytotoxic effect, thereby reducing viability to about 10% (P-value <0.000) (Figure 6). Additionally, the concentration of Phenelzine was 11.16 µM that inhibited 50% of the growth of LPS (100 ng/ml) pre-treated RAW264.7 cells over 24 hours compared to the control (IC50) (Figure 7).

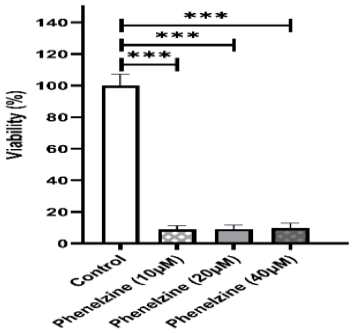

Figure 5. Viability of RAW264.7 cells after 24 hours of treatment with 10 µM, 20 µM, and 40 µM phenelzine showed a severe reduction. The mean viability ± SD was calculated using the formula (OD test/OD control × 100) and analyzed using the Kruskal-Wallis test (* p <0.05, ** p <0.0), *** p <0.001).

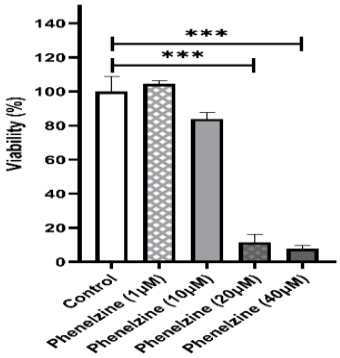

Figure 6. Viability of RAW264.7 cells after 24 hours of treatment with 1 µM and 10 µM Phenelzine showed no significant change, while 20 µM and 40 µM concentrations caused a severe reduction in the viability. All groups were pre-treated with LPS (100 ng/ml). The mean viability ± SD was calculated using the formula (OD test/OD control × 100) and analyzed using the Kruskal-Wallis test (* p <0.05, ** p <0.0), *** p <0.001).

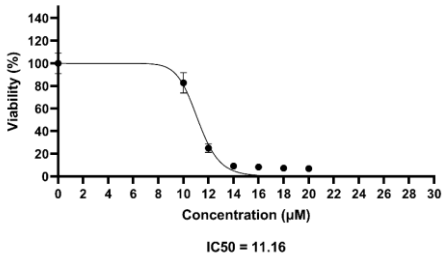

Figure 7. Phenelzine's IC50 in LPS -stimulated RAW264.7 cells over 24 hours (100 ng/ml). The IC50 was determined to be 11.16 μM. The mean viability ± SD was calculated using the formula (OD test/OD control × 100) and plotted accordingly. The IC50 value was obtained using non-linear regression analysis with a variable slope.

Discussion

Considering the known reciprocal link between inflammation and depression, this study aims to explore the effects of Phenelzine and serotonin on RAW264.7 macrophages using the MTT assay. To this aim, we assessed whether different concentrations of Phenelzine, serotonin, and 5-HIAA influence viability of RAW264.7 cells. Our findings indicate that treatment with serotonin leads to a concentration-dependent increase in cell proliferation, which is consistent with previous studies (8,29). Serotonin, through the activation of the 5-HT2B receptor, enhances the proliferation of various cell types via phosphorylation of Gαq and Src and production of growth factors such as insulin-like growth factors, TGFβ1, CTGF, FGF2, and TGFα (29).

Furthermore, another study demonstrated that 5-HT2B receptor in macrophages prevents the degeneration of mononuclear phagocytes in amyotrophic lateral sclerosis (ALS) (30). Additionally, treatment of RAW264.7 cells with 5-HIAA and the product of serotonin metabolism by MAO at concentrations of 1μM, 10μM, and 100μM did not show any notable effect on cell viability.

Our research reveals that although Phenelzine at 10 μM concentration notably decreases the viability of RAW264.7 cells, pre-treatment with 100 ng/ml of LPS can significantly counteract this cytotoxic effect and enhance cell survival. A study showed that tolerance and protection against high concentrations of LPS are induced in PC12 cells pre-treated with LPS for 12 hours at a concentration of 3 μg/ml, thus preventing cell cycle arrest and apoptosis (31).

Conclusion

Our study suggests that although treatment with Phenelzine increases serotonin availability, there are other mechanisms that may decrease RAW264.7 cell viability and counteract the enhanced proliferation effect in a concentration-dependent manner. Phenelzine, an antidepressant prescribed for patients with neurological disorders who may also be susceptible to immune-related conditions and cancer, can impact macrophage cell viability and consequently innate immune response. Based on the findings of the present study, inflammatory conditions and RAW264.7 cell treatment with 100 ng/ml LPS can diminish the cytotoxic impact of Phenelzine. Nevertheless, investigating Phenelzine's influence on the immune system, especially in those prone to immune conditions and cancer, can lead to better treatment approaches for such patients.

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Ethical statement

This study was performed in line with the principles of the Declaration of Helsinki. Approval was granted by the Ethics Committee of Golestan University of Medical Sciences (May 29 2021/IR.GOUMS.REC1400.369.).

Conflicts of interest

The authors have no relevant financial or non-financial interests to disclose.

Author contributions

Conceptualization, project administration, Homa Davoodi, Supervision, methodology, review and editing Saeed Mohammadi; project administration, Afifeh Jaefari. All authors read and approved the final version of the manuscript.

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