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The Effects of Thymosin Alpha-1 on Macrophages: A Cytological and Anti-Inflammatory Study

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Abstract

Thymosin alpha 1 (Tα-1) is a naturally occurring substance synthesized by the thymus tissue, known to activate various immune system cells. It has been reported to increase the production of Natural Killer cells, CD4 and CD8 cells, and cytokines such as IL-2, IL-3, and IFNγ. Furthermore, it plays a vital role in regulating immunity, inflammation, and tolerance. Its effect on the immune system is exerted via its modulatory action on innate immune system cells, thereby functioning as an endogenous regulator for both inflammatory and adaptive immune systems. In this study, we sought to evaluate the cytological and anti-inflammatory effects of Tα-1 on RAW 264.7 cells. The cytological effects of Tα-1 were assessed using the MTT assay, with the IC50 value against RAW 264.7 cells determined to be 368.105 ug/ml. The morphological observations made at various concentrations of Tα-1 showed increased cytotoxicity and decreased cell density of RAW 264.7 cells with increasing test concentration. Furthermore, the anti-inflammatory effects of Tα-1 were evaluated by analyzing the nitric oxide (NO) production in RAW 264.7 cells. Treatment with the test items at concentrations ranging from 7.813 to 31.25 ug/ml showed a dose-dependent reduction in NO production compared with the control group. These findings suggest that Tα-1 may have the potential as an anti-inflammatory agent in the treatment of various diseases associated with inflammation.

Keywords: *Thymosin alpha-1; Immune system; Interleukins; Natural killer cells; inflammation.*

1. Introduction

Thymosin alpha-1 (Tα-1), a 28 amino acid glycoprotein, working as an important modulator of immunological responses and released from the thymus. While the thymus has the highest concentrations of T-1, it has also been detected in the kidney, brain, spleen, lungs, and other tissues. T-1 is a physiological regulator that enhances immunological cell activity and is used to manage an array of disorders in which the immune response is impaired or inefficient. Its immunoregulatory actions include increasing T-cell generation, increasing natural killer cell activity, and decreasing inflammatory response. Additionally, T-1 has been used for an array of diseases caused by immunological dysfunctions, including hepatitis B and HCV, many cancers, severe sepsis, and as an adjuvant to enhance vaccinations [1].

Tα-1's cellular method of action has been found to have both immune-modulating and direct-acting properties. It engages with cytoplasmic Toll-Like Receptors and, in organic solvents, can fold into a coiled helix, enabling it to penetrate the cell membrane on its own [2]. Tα-1's immunomodulatory effects are achieved via its impact on innate immune system cells, such as dendritic cells and macrophages, and also cells of the adaptive system, like T-cells and B-cells. Tα-1 has been demonstrated to promote the release of cytokines such as IL-2, IL-4, IL-6, and IFN, all of which play

important roles in the homeostasis of immune responses. In conclusion, Tα-1 works as an important protein hormone that regulates the immune system's functions. Its ability to stimulate immune cell activity and reduce inflammation has made it a promising therapeutic agent for treating a range of diseases. Further research is necessary to uncover Tα-1's full range of mechanisms of action, which may lead to the development of new treatment options for immunological disorders. Tα-1 has been observed to positively impact stem cell and immune cell production. Specifically, it has been shown to stimulate the production of mature T lymphocytes, particularly CD4 cells, by encouraging the proliferation of stem cells. Tα-1 has also been used to promote thymopoiesis in human CD34 stem cells, which has led to increased production of interleukin-7, an essential cytokine for thymocyte development [3].

In addition, Tα-1 has been found to boost the physiological characteristics of Natural Killer (NK) cells in in-vivo system and also in HIV-positive individuals. This is significant, as NK activity tends to decrease in individuals with hepatitis C infections [4-8]. Furthermore, Tα-1 can promote the production of Th1 cytokines like IFN, IL-2, and IL-3, as well as the expression of IL-2 receptors, leading to a Th1 immunological response which is also linked with strong antiviral activity [9]. Conversely, a Th2 immunological response which is linked with the continuation of infections, and the production of Th2 cytokines may serve as a viral defense mechanism. Tα-1 has been found to significantly increase the production of IL-2 hepatitis-C patients, and this impact was more pronounced than that of IFN treatment alone [10-13]. When used in conjunction with IFN therapy, Tα-1 has shown promise in enhancing T-cell proliferation essential for long-lasting removal of the hepatitis-C virus, while simultaneously suppressing IFN-linked Th2 responses [14-16].

Understanding the effects of Tα-1 on macrophages is essential for developing new treatments for diseases characterized by inflammation, such as autoimmune diseases and cancer. Here, we explore the cytological and anti-inflammatory impacts of Tα-1 on macrophages. We hypothesize that Tα-1 will enhance the anti-inflammatory response of macrophages and provide new insights into the potential therapeutic applications of Tα-1 [17-18].

2. Materials And Methods

Cytotoxic assay Methodology:

RAW 264.7 were bought from the NCCS Pune, India. Cells were grown in log stage in “Dulbecco's modified eagle medium (DMEM)” supplemented with 10% (v/v) thermal attenuated “fetal bovine serum (FBS)”, “100 U/mL penicillin”, and “100 ug/mL streptomycin”. The “MTT (3-(4,5-dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide)” test was implemented to assess the cytotoxicity of the substance against the RAW 264.7 cell line [19]. The grown cell from the cultured plates were seeded in 96-well microtiter plate (1x107 cells/well), cultivated for two days at 37°C in a 5% CO2 incubator, and allowed to grow to 70-80% confluence. After being subjected to various quantities of materials, the media was replenished, and the cells were subsequently cultivated for 24 hours. After 24 hours, the morphological changes of untreated (controlled) and treated cells were analysed and photographed using a computerized inverted microscope at 20X magnification.

$$Cell\ viability\ (\%) = \left(\frac{Absorbance\ of\ sample}{Absorbance\ of\ control}\right) X100$$

Anti-inflammatory assay:

RAW 264.7 cells were housed in a humid chamber with 95% air at 37°C and 5% CO2. 1x100,000 cells/mL were pre-incubated for 1 hour with different testing item concentrations before being stimulated for 24 hours at 37°C with 1 ug/mL LPS in the medium. Monitoring the degree of NO generation by measuring the nitrite content in the culture media. To do this, the medium was combined with the Griess reagent system. After 10 minutes of incubation, the absorption spectrum was recorded at 540 nm. The nitrite level was determined using a sodium nitrite calibration curve as a benchmark [20-22].

3. Results and Discussion

Cytotoxic assay:

The MTT test was used to determine the sample's cytotoxicity. After treating with various concentrations of Tα-1, it was discovered that the drug concentrations ranging from 15.62 ug/mL to 31.25 ug/mL were those with the highest cell viability. After the results, it was found that the IC50 value of Tα-1 against RAW 264.7 cells were found to be 368.10 ug/ml.

Table 1. Cytotoxic activities of thymosin alpha-1 RAW 264.7 cells

Concentrations (ug/mL)	Absorbance			Average	Cell Viability (%)	Inhibition (%)
	I	II	III			
Control	0.42	0.42	0.42	0.42	100	0
15.62	0.41	0.41	0.40	0.41	97.71	2.28
31.25	0.40	0.39	0.40	0.39	94.25	5.74
62.5	0.36	0.37	0.38	0.37	88.50	11.49
125	0.35	0.33	0.34	0.34	81.02	18.97
250	0.27	0.27	0.29	0.28	66.14	33.85

Morphological observations of different concentrations were also observed and recorded in the supplementary file. The observations show that increasing cytotoxicity and decreasing cell density of RAW 264.7 cells with an increase in test concentration treatment. Morphological changes are shown in the pictures given below in figure 1.

Anti-inflammatory assay:

This cell line is a commonly used model for studying macrophage biology and immune function. The evaluation of the anti-inflammatory effects of the Tα-1 was done by assessing the nitric oxide (NO) release in RAW 264.7 cell line. The evaluation of the anti-inflammatory effects of Tα-1 on NO production in RAW 264.7 cells was a key aspect of our study. Various concentrations of sample given below in the table-2 was use to evaluate the production of nitric oxide. It was found that the test items at concentrations ranging from 7.81 to 31.25 ug/mL showed a dose-dependent reduction of NO production compared with the control.

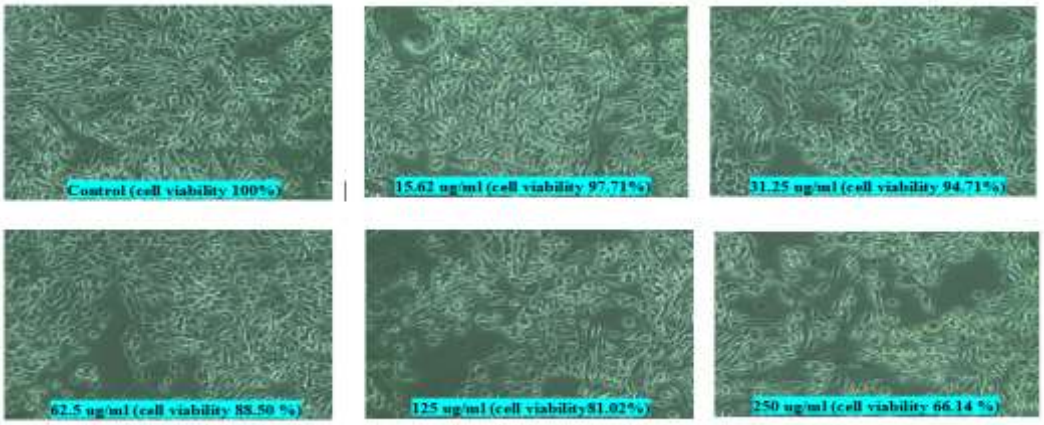

Figure 1. Dose-dependent cytotoxicity effect of Tα-1 and morphological changes in the RAW 264.7 cells

Table 2. Nitric oxide release after the treatment of LPS and different concentrations of Tα-1 in RAW 264.7 cells

Concentrations (ug/mL)	Absorbance			Average	Nitric Oxide (uM/l)
	I	II	III		
Untreated	0.08	0.08	0.07	0.07	7.76
LPS Induced	0.25	0.26	0.26	0.26	40.04
LPS + 7.81	0.24	0.23	0.23	0.23	35.48
LPS + 15.62	0.22	0.21	0.21	0.21	31.91
LPS + 31.25	0.16	0.15	0.15	0.15	21.80

NO is a key mediator of inflammation and is produced by macrophages in response to various stimuli. Our results indicate that Tα-1 treatment leads to a dose-dependent reduction in NO production in RAW 264.7 cells (table 1, figure 2). This finding suggests that Tα-1 has significant anti-inflammatory effects in macrophages and may be an effective therapeutic agent for the treatment of inflammatory diseases. Tα-1's ability to lower NO production might be attributed to its capacity to modulate the functioning of inductive nitric oxide synthase (iNOS), the enzyme accountable for NO production in immune cells. Recent research has demonstrated that Tα-1 can suppress iNOS expression as well as activity, resulting in a reduction in NO generation.

The RAW 264.7 cell line is a widely used model for studying macrophage biology and immune function. In our study, we investigated the effects of Tα-1 on RAW 264.7 cells and observed significant changes in their cytological and anti-inflammatory properties.

Firstly, we found that Tα-1 treatment increased the viability of RAW 264.7 cells. This indicates that Tα-1 may protect macrophages, promoting their survival and preventing cell death. This effect may be due to Tα-1's ability to enhance the expression of anti-apoptotic genes or through the modulation of cellular metabolism. Secondly, we observed that Tα-1 treatment reduced the creation of pro-inflammatory cytokines like TNF-α and IL-6 and increases proinflammatory cytokines synthesis and secretion like IL-10.

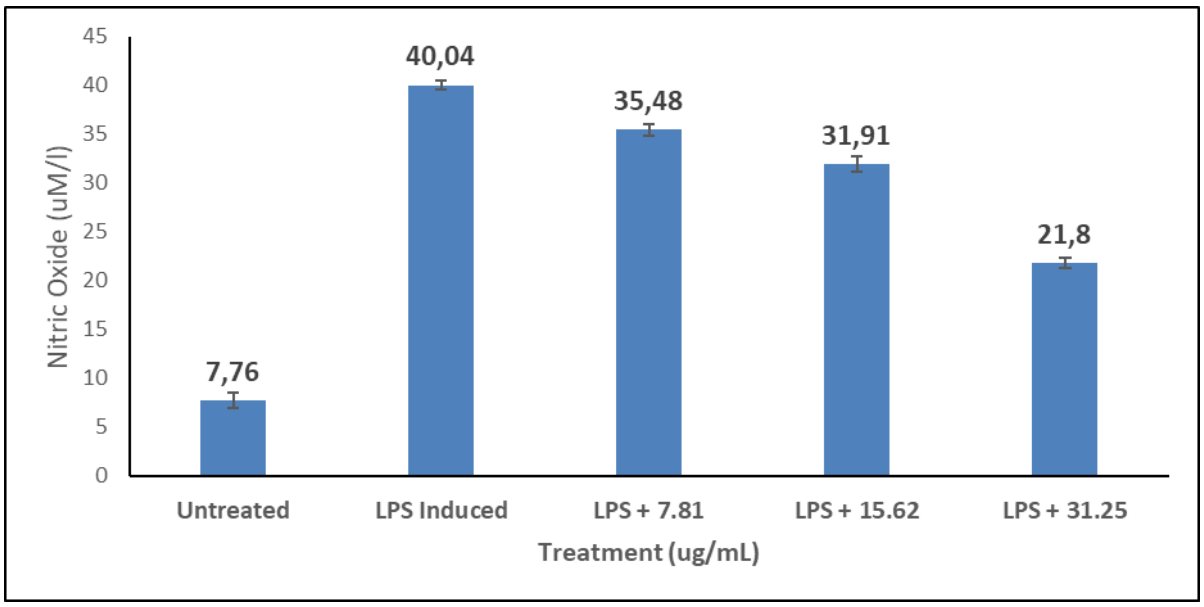

Figure 2. Nitric oxide release after the treatment of LPS and different concentrations of Tα-1 in RAW 264.7 cells

This is consistent with previous studies demonstrating Tα-1's anti-inflammatory effects in various cell types, including macrophages. Tα-1 may achieve this by modulating the activity of transcription factors such as NF-κB, which are known to be involved in the regulation of pro-inflammatory gene expression. The ability of Tα-1 to enhance IL-10 production suggests that it might be an efficient medicinal means in the management of inflammatory diseases. Overall, our results suggest that Tα-1 has significant cytological and anti-inflammatory effects on RAW 264.7 cells, highlighting its potential as a remedial agent in the management of inflammatory diseases. However, further research is needed to investigate the underlying mechanisms of Tα-1's effects on macrophages and to evaluate its efficacy in treating specific diseases.

Tα-1 is a naturally occurring biological modulator that has been shown to enhance immune cell activity and increase the production of cytokines such as IL-2, IL-3, and IFNγ (10-13). Additionally, Tα-1 is a critical regulator of immunity, inflammation, and tolerance. Its immunomodulatory effects are achieved through its action on the innate immune system cells, making it a vital endogenous regulator for both the inflammatory and adaptive immune systems.1 This study aimed to investigate the cytotoxicity and anti-inflammatory effects of Tα-1. In particular, we sought to determine the impact of increasing Tα-1 concentration on nitric oxide production in the anti-inflammatory assay. Our findings indicate that increasing the concentration of Tα-1 reduces the production of nitric oxide, highlighting its anti-inflammatory effects. Overall, our results contribute to the growing body of evidence demonstrating the beneficial effects of Tα-1 on the immune system and its potential as a therapeutic agent in the management of inflammatory diseases. Further research is needed to elucidate the mechanisms underlying its anti-inflammatory effects and to evaluate its efficacy in treating specific diseases.

4. Conclusion

In conclusion, our study has provided evidence that Tα-1 has significant cytological and anti-inflammatory effects on macrophages. Our findings suggest that Tα-1 enhances the anti-inflammatory response of macrophages, reducing inflammation and potentially mitigating the severity of diseases characterized by inflammation, such as autoimmune diseases and cancer. Overall, the evaluation of Tα-1's effects on NO synthesis and secretion provides important insights into its anti-inflammatory properties and potential as a therapeutic agent in managing inflammatory diseases. Further investigation

is ought to investigate the underlying mechanisms of Tα-1's effects on macrophages and to evaluate its efficacy in treating specific diseases.

Our study showed that Tα-1 can modulate macrophage activity and improve their function, highlighting its potential as a therapeutic agent. Further research is needed to investigate the mechanisms underlying Tα-1's effects on macrophages and to evaluate its efficacy in treating specific diseases. Nonetheless, our study provides important insights into the immunomodulatory effects of Tα-1 and its promise as a medicinal agent in the controlling agents of inflammatory diseases.

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Conflicts of Interest

The authors declare no conflict of interest.

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