

**Histological and Immunohistochemical Study
on the Effect of Ghrelin Hormone on Thymic
Atrophy Induced by Acute Stress in Male
Mouse**

Thesis

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Dedication

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List of Abbreviations

Abb.	Full term
<i>4CP2</i>	<i>4 Chlorophenol 2</i>
<i>APC</i>	<i>Antigen Presenting Cell</i>
<i>BCL2/BAX</i>	<i>B Cell Lymphoma 2/B Associated Protein</i>
<i>CCR7</i>	<i>Chemokine Receptor</i>
<i>CD</i>	<i>Cluster of Differentiation</i>
<i>CD26L</i>	<i>L Selectin</i>
<i>CNS</i>	<i>Central Nervous System</i>
<i>cTEC</i>	<i>Cortical Epithelial Reticular Cell</i>
<i>DAB</i>	<i>Diamino Benazidine Tetrahydrochloride</i>
<i>DC</i>	<i>Dendritic Cell</i>
<i>DN</i>	<i>Double Negative</i>
<i>DP</i>	<i>Double Positive</i>
<i>DPX</i>	<i>Di-N-Butyle Phthalate in Xylene</i>
<i>E/M</i>	<i>Electron Microscope</i>
<i>FOXP3</i>	<i>Forkhead Box P3</i>
<i>GC</i>	<i>Glucocortic</i>
<i>GHS-R</i>	<i>Growth Hormone Secretagogue Receptor</i>
<i>GHSR1a</i>	<i>Ghrelin Receptor</i>
<i>GHSs</i>	<i>Growth Hormone Secretagogues</i>
<i>GOAT</i>	<i>Ghrelin-O-Acyltransferase</i>
<i>IL</i>	<i>Interleukin</i>
<i>INF-γ</i>	<i>Interferon Gamma</i>
<i>IP3</i>	<i>Inositol 1,4,5-Trisphosphate</i>
<i>LSD</i>	<i>Least Significance Difference</i>
<i>MHC</i>	<i>Major Histocompatibility Molecule</i>
<i>MKP-1</i>	<i>Mitogen-Activated Protein Kinase Phosphatase-1</i>
<i>mRNA</i>	<i>Messenger Ribonucleic Acid</i>
<i>mTEC</i>	<i>Medullary Epithelial Reticular Cell</i>

List of Abbreviations (Cont...)

Abb.	Full term
<i>NK</i>	<i>Natural Killer</i>
<i>PBMCs</i>	<i>Peripheral Blood Mononuclear Cells</i>
<i>PBS</i>	<i>Phosphate Buffered Saline</i>
<i>ROS</i>	<i>Reactive Oxygen Species</i>
<i>SD</i>	<i>Standard Deviation</i>
<i>SP</i>	<i>Single Positive</i>
<i>SPSS</i>	<i>Statistical Package for the Social Sciences</i>
<i>TCR</i>	<i>T-cell Receptor</i>
<i>TEC</i>	<i>Epithelial Reticular Cell</i>
<i>TGF-β</i>	<i>Transforming Growth Factor Beta</i>
<i>TNF</i>	<i>Tumor Necrosis Factor</i>
<i>Treg</i>	<i>T regulatory</i>
<i>VEGF</i>	<i>Vascular Endothelial Growth Factor</i>
<i>X/A-like cells</i>	<i>X-Unknown Factor, A Cells of Pancreas</i>
<i>α cells</i>	<i>Alpha Cells</i>
<i>β cells</i>	<i>Beta Cells</i>
<i>ε cells</i>	<i>Epsilon Cells</i>

Abstract

Background: Exposure to stress down regulates the immune system. Thymus gland is sensitive to stress. Ghrelin hormone secreted by the stomach has an immune-stimulatory effect.

Aim of the Work: was to study the effect of immobilization stress on mouse thymic population and the possible protective role of Ghrelin.

Material and Methods: 40 animals were divided into four groups (10 mice each). Group I was considered as control group. Group II was injected with a 100 µg/kg of ghrelin intraperitoneally. Group III was immobilized by stress restraint test. Group IV received 100µg/kg intraperitoneally prior to the stress restraint test. Thymi of mice were removed and processed for haematoxylin and eosin, immunohistochemical staining for caspase 3 and electron microscopic analysis. Finally morphometric and statistical analysis were performed.

Results: Acute stress resulted in significant decrease in thymic weight. Atrophy of thymic lobule with marked fatty and mononuclear cellular infiltration was detected.

Marked decrease in cellularity of thymic cortex was detected and confirmed by significant increase in caspase 3 positive cells. Medulla showed proliferation of epithelial reticular cells with cystic degeneration in Hassall's corpuscle.

In Ghrelin protective group, the thymus regained normal histological structure with significant decrease in caspase 3 positive cells.

Conclusion: Stress resulted in loss of double positive thymocytes to the periphery. Extra thymic T cells were defective, non functional and auto-reactive. Ghrelin allowed activation of surviving thymocytes and prevented apoptosis.

Keywords: Thymus, Stress, Ghrelin, Mice, Histology, Electron Microscope

INTRODUCTION

Our constant exposure to environmental stressors has become a major problem in our society. The increased impact of daily stressors has taken its toll on our immune system. Stress may take several forms and its effect depends on duration, intensity and nature of the effector (*Majumdar and Nandi, 2018*).

The thymus gland is a crucial organ for homeostatic maintenance of the peripheral immune system. It is the mediastinal tissue responsible for proper T cells development and education. It is the site where thymocytes are extensively differentiated into functional types for migration to the periphery and building of an effective and self-oriented immune system (*Liu and Ellis, 2016*).

Thymic atrophy is a complication that results from exposure to many factors including drugs, cancer therapies and microbial invasion. The acute stress associated thymic atrophy can have a devastating effect on the host's immune system. The loss of the natural ability to produce an adequate population of naïve T cells exposes the body to severe danger even from minor pathogens (*Lee et al., 2016*).

Many efforts have been made to increase our understanding of thymus biology, microenvironment and development. Provisional therapies aiming at re-establishing an

adequate and effective T cell production in patients with a defective immune system is mandatory (*Deboer et al., 2012*).

Ghrelin is a 28 amino-acid peptide hormone secreted by the stomach. It acts at the Ghrelin receptor in multiple tissues throughout the body, exhibiting broad effects potentially beneficial as a treatment in human disease states (*Zhou et al., 2018*).

Recently Ghrelin was reported to modulate apoptotic signaling in several cell types, such as cardiomyocytes, endothelial cells, adipocytes, adrenal zona glomerulosa cells, pancreatic β -cells, osteoblasts and hypothalamic neurons (*Baatar et al., 2011*).

AIM OF THE WORK

The aim of our work was to study the effect of stress on thymic architecture, cellularity and function and to prove the possible protective effect of Ghrelin hormone on stressed thymus of male mouse.

THYMUS

Embryology

The thymus is a bilobed organ that is located in the central compartment of the thoracic cavity, on top of the heart and behind the sternum. It is the primary site of T-lymphocyte development. It is essentially an epithelial organ, containing many developing lymphocytes, that is surrounded by a mesenchymal capsule (*Haley, 2003*).

The thymus has two different origins for the lymphoid thymocytes and the thymic epithelial cells, hence the name lymphoepithelial organ. It develops from the endoderm of the 3rd and to a lesser extent the 4th pharyngeal pouches and surrounding mesenchyme. During the sixth week of gestation, the endoderm of the 3rd pharyngeal pouch forms a sacculization called thymic primordia which soon is invested by a layer of ectoderm. By the 8th week of gestation, it migrates caudally to occupy their position in the anterosuperior mediastinum (*Elmor, 2006*).

After migration, the thymic endodermal-derived epithelial cells form a reticular meshwork. The surrounding mesenchyme forms the surrounding capsule which down grows to form trabeculae dividing the organ into numerous lobules. By the 10th week of gestation, small lymphoid cells from the fetal liver and bone marrow migrate to the thymus, which differentiates into a

cortex and a medulla. Small tubular structures formed of epithelial cells (also called medullary duct epithelium) give rise to Hassall's corpuscles (*Bleul et al., 2006*).

The thymus is the first lymphoid organs to be formed and grows soon after birth in response to exposure to postnatal antigen and the increasing need for mature T cells. Thymus immunological function varies according to genetic factors affecting the age of onset, rate and magnitude of thymic response. It reaches maximal size by the age of sexual maturity and then involutes gradually by aging (*Farley et al., 2013*).

Histological structure of the thymus

The thymus can be histologically divided into lobules formed of a denser outer region the cortex and a looser inner zone the medulla. Both regions are formed of a distinct population of epithelial reticular cells and thymic lymphocytes (*Pearse, 2006*).

a) The capsule: Thymus has thin connective tissue capsule surrounding each lobe. It gives rise to the septa that divide the thymus into interconnecting lobules of variable sizes and orientation. There is no distinct sublobulation in the thymus of mouse. The capsule is composed of an outer and inner layer of collagen and reticular fibres, between which are occasional clusters of lymphocytes (*Gui et al., 2012*).