**Effect of Acute Stress on Immune Cell Counts and the Expression of Tight Junction Proteins in the Duodenal Mucosa of Rats**

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**Background/Aims:** Duodenal immune alterations have been reported in a subset of patients with functional dyspepsia (FD). The aim of this study was to investigate the effect of acute stress on immune cell counts and the expression of tight junction proteins in the duodenal mucosa. **Methods:** Twenty-one male rats were divided into the following three experimental groups: 1) the nonstressed, control group, 2) the 2-hour-stressed group, and 3) the 4-hour-stressed group. Eosinophils, mast cells and CD4+ and CD8+ T lymphocytes in the duodenal mucosa were counted. The protein and mRNA expressions of occludin and zonula occludens-1 (ZO-1) were examined. **Results:** Eosinophils, mast cells and CD8+ T lymphocyte counts did not differ between the stressed and control groups. The number of CD4+ T lymphocytes and the protein and mRNA expressions of occludin and ZO-1 were significantly lower in the 4-hour-stressed group compared with the control group. The plasma adrenocorticotrophic hormone and cortisol levels of the 4-hour-stressed group were significantly higher than those of the control group. **Conclusions:** Acute stress reduces the number of CD4+ T lymphocytes and the expression of tight junction proteins in the duodenal mucosa, which might be associated with the duodenal immune alterations found in a subset of FD patients. *(Gut Liver 2013;7:190-196)*

**Key Words:** Duodenum; Lymphocytes; Stress; Tight junction protein

**INTRODUCTION**

Functional dyspepsia (FD) is a heterogeneous disorder characterized by the presence of recurrent or persistent symptoms thought to originate in the gastroduodenal region without any organic, systemic or metabolic disease that is likely to explain the symptoms. The pathophysiology of FD is not completely understood yet. Although the stomach is traditionally believed to be mainly responsible for dyspeptic symptoms, recent studies have shown the possibility of duodenal involvement in the pathophysiology of FD. Abnormal motor and sensory responses to duodenal acid or lipids have been demonstrated in patients with FD. Alterations in the number of immune cells have been observed in the duodenal mucosa of patients with FD. Since cytokines, chemokines, and neuroactive chemicals released from immune cells may affect gastrointestinal motility and sensitivity, duodenal immune cell alterations may be implicated in the pathophysiology of FD. However, how these changes occur is unclear yet and remains to be explored.

FD is known to be related to stress. Experimental studies provide evidence that acute psychological stress exerts an inhibitory effect on the duodenal and gastric motility. In addition, stress is found to contribute to inflammation by altering epithelial permeability. Immunologic alterations found in the duodenal mucosa of FD patients might be associated with stress. So, we hypothesized that stress affect immune cells and epithelial tight junction (TJ) proteins in the duodenal mucosa.

Thus, the aim of the present study was to investigate the effect of acute stress on immune cell counts and the expression of TJ proteins in the duodenal mucosa of rats.

**MATERIALS AND METHODS**

1. **Animals**

Twenty-one adult male Sprague Dawley rats (250 to 300 g), aged 16 to 18 weeks were used for the present study. All rats were acclimated for 7 days before experimentation and allowed free access to food and water. Rats were kept on a 12-hour

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light:dark cycle and isolated from environmental stressors (e.g., noise) as much as possible. The animals were housed in pairs in cages and kept in a temperature-controlled room (21°C ± 1°C). The rats were handled daily for a week by the same examiner. All protocols were approved by the Institutional Animal Care and Use Committee at Ajou University School of Medicine (AMC-103).

2. Experimental protocols
Rats were divided into three experimental groups with seven rats per treatment group: 1) the nonstressed group, 2) the 2-hour-stressed group (rats were assigned to receive water-immersion restraint stress for 2 hours), and 3) the 4-hour-stressed group (rats were assigned to receive water-immersion restraint stress for 4 hours). Rats in those separate groups are identical in sex, weight and age. In all stress sessions, the total body of the animal from head to lower hind limbs was tightly placed in wire cages, and the entire body except the head was immersed vertically to the level of the xiphoid process in a water bath maintained at 19°C ± 1°C. The stress session was performed just one time. Control rats were placed in their home cages without exposure to any restraint stress. Immediately after completing the experiments according to the protocol, all rats were sacrificed by stunning and posterior exsanguination. Blood and tissues were collected immediately after sacrifice.

3. Histological evaluation
Mucosal tissues were obtained from the duodenum, immediately fixed in formalin and embedded in paraffin wax. Serial sections were stained with hematoxylin and eosin for routine histological evaluation under light microscopy. Eosinophils were counted in five nonoverlapping high power fields (HPF; final magnification, ×400) (Fig. 1A). Toluidine blue staining was performed to identify mast cells (Fig. 1B). Mast cells located in

![Figure 1](image-url)