Ultrastructural Changes on the Synovial Membrane of Rat Temporomandibular Joint under Mild Restraint and Cold Stresses


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I. INTRODUCTION

Nowadays, everyone seems to be talking about stress. You hear it not only in daily conversation but also through television, radio, the newspapers and the constantly increasing number of conferences, stress centers and university courses that are devoted to the topic. Stress, one type of the interaction, is the nonspecific response of the body to any demand. In the course of daily life, there is a continuous ongoing interaction between the individual and environment. Stress can have an effect on human body which is apt to maintain homeostasis and modulate three main systems such as autonomic nervous system, the hormonal system and immune response system. Then, it can affect various organs at cellular and molecular levels starting with cellular responses to injury: cell adaptive response, cell stress response (production of stress protein), reversible cell injury and ultimately irreversible cell injury such as apoptosis (programmed cell death). In that way, the extremely complex self-regulating system can alter the internal functions down and cause various diseases. Modern medicine has relatively little interest in cognitive functions, psychological techniques, or the effects of nuances of belief, faith, self-suggestion, or yogic exercises on the internal functions in treatment regimens. On the other side of the therapeutic coin, psychology has fostered few formal applications for medicine. There are, however, emerging concepts of the cause and treatment of emotional, psychosomatic, and related problems, now popularly designated as stress-related problems, that are directed toward both psychophysiological relationships in health and illness and the influences of higher-order mental function. So the term ‘psychoneuroimmunology’ has developed in order to elucidate the relationship between psychosocial stress and diseases. There are many stress-related symptoms and diseases in the orofacial tissue such as lichen planus, aphthous
stomatitis, geographic tongue, recurrent herpes labialis, xerostomia, halitosis, burning mouth syndrome, muscle tension headache, atypical odontalgia and temporomandibular disorders (TMD).  

Among the stress-related orofacial diseases, although increasing attention has been recently addressed to TMD, the pathologic mechanism of TMD induced by stress remains vague. Like most joint diseases, TMD is associated with alterations in the synovial membrane. It is essential to observe temporomandibular joint (TMJ) synovial cell layers in respond to a broad variety of stress condition because synovial membrane plays an important role in maintaining normal joint physiology while they can be easily affected by stress like other membrane boundaries between fluid and tissue compartment such as salivary glands, esophagus, vagina and ovarian granulosa cells. In order to have a better understanding of the pathologic mechanism in TMD, emphasizing the significant role of stress in the disease, the present study was performed to investigate the cellular adaptability and the ultrastructural change of TMJ synovial membrane under physical and psychological stress.

II. MATERIALS AND METHODS

1. Experimental animals

Sprague-Dawley rats (8-week-old, 323-367 g/bw) were purchased from Dae-Han Experimental Animal Research Center, Seoul, Korea. They were maintained at 20-23°C and fed ad libitum on a normal laboratory diet. The rats were divided into 3 groups: 1) Normal control group; 2) Mild restraint stress group; 3) Cold stress group: the rats of restraint stress group were placed in the stress cage permitting slight movement throughout the period of experiment and the rats of cold stress group were immersed in cold water (4°C) for 6 min once a day throughout the experiment. All the animals were then sacrificed at day 1, 3, 5, and 7 day of the experiment and the synovial membrane of temporomandibular joint(TMJ) were excised immediately and stored in the glutaraldehyde in phosphate buffer (PB) after rinsed in PB.

2. Electron Microscopy

The tissues were rinsed in 0.1 M cacodylate buffer 3 times 10 min each, postfixed in 1% osmium tetroxide for 90 min, and rinsed in 0.1 M malate buffer 3 times 5 min each. They were prestained with 1% uranyl acetate for 90 min and washed in 0.1M malate buffer 3 times 5 min each, and dehydrated through an ascending series of ethanol concentration (50% to 100%, 15 min each). And they were placed in 100% ethanol and propylene oxide (1:1) for 45 min, propylene oxide for 45 min, and propylene oxide and epon (1:1) for 1 hr. After then, they were placed in epon in a vacuum oven overnight, embedded with fresh epon which was polymerized at 60°C for 3 days. The embedded tissues were cut with a diamond knife 50 nm thick and stained with uranyl acetate and lead citrate. The tissues were observed by the transmission electron microscopy (JEM-1010, JEOL Ltd, Japan).

III. RESULTS

In the normal control group, many rough endoplasmic reticulum(rER), mitochondria were observed in a normal synovial cell.

In the mild restraint stress group, in lapse of time, according to the mild restraint stress, ultra-structural changes appeared gradually.

1. Mitochondria were partially condensed, and the number and the size of mitochondria were decreased gradually.  
2. rERs were gradually dilated with irregular shape.  
3. Double layer of nuclear envelope were slightly corrugated at day 7.

In the cold stress group, in lapse of the time, according to the cold stress, ultrastructural changes are as follows.
1. Mitochondria had shown slight swelling shapes, and were decreased in number at day 1, 3, but again increased in number at day 5, 7, with very sound shapes.

2. The lamellated structures of the rER were changed to dilated pattern at day 1, 3, but were gradually recovered and activated at day 5, 7.

3. Nucleus was almost intact in all cold stress group during all period of the experiment.
IV. DISCUSSION

Stress is defined as a complex dynamic condition in which normal homeostasis is disturbed. The physiological stress response comprises three main systems such as autonomic nervous system, hormonal system and immune response system. The systems are extremely complex and self-regulating, involve multiple causal factors, and key basic functions of them are surveillance, mobilization of resources and feedback. Depending on the type and degree of stress, the tissue response to it could be physiologic or pathologic. If stress exceeds adaptability of the tissue, it may affect various organs starting with alterations at cellular and molecular levels by cellular responses to injury: cell adaptive response, cell stress response (production of stress protein), reversible cell injury, and ultimately irreversible cell injury such as apoptosis. (programmed cell death)

This distinction identifies stressors as ‘physical’ or ‘psychological.’ Physical stressors have direct contact with an organism and most common form for the experiment is a forced cold swim. In contrast, psychological stressor defined as the activation of the central nervous system without any contact with the organism. Common stressors of this type include immobilization or restraint. Two types of stressors have been widely used to illustrate the influence of stress on the body. Stresses may be further classified into psychological stress, social stress, economic stress and physiological stress. One of the most important pathways involved in stress is HPA axis (Hypothalamo–pituitary adrenal axis). (AP: Harris GW 1948) When stress activates the HPA axis, glucocorticoids are massively secreted by adrenal cortex. the HPA axis is stimulated by corticotrophin-releasing factor(CRF), resulting in a down-regulation of immune function. Stress–induced alteration in endocrine function and potential pathophysiologic effects of endocrine change lead to a depletion of subject’s ability to resist stress, which ultimately leads to the disease process.

The HPA axis releasing endogenous glucocorticoid is thought to play a role in the stress responses including apoptosis of thymocytes and mature T cells and inhibiting the trafficking of immune cells. Stress affects the numbers and functions in monocytes, lymphocytes and CD4/CD8
ratio and impaired immune response including antibody production, natural killer activity and lymphocyte responses to mitogen stimulation.\textsuperscript{13,14} Kuyawama et al. demonstrated that water-immersion restraint stress result in an increase in gastric epithelial cell loss and macroscopic mucosal injury.\textsuperscript{15} Immobilization stress provokes an elevation of serum corticosterone concentrations which causes the decline in testosterone concentrations.\textsuperscript{16} Chronic stress causes an epithelial barrier defect and epithelial mitochondrial damage, in parallel with mucosal mast cell hyperplasia and activation according to Santos J et al.\textsuperscript{17}

In multicellular organisms, homeostasis is maintained through a balance between cell proliferation and cell death.\textsuperscript{18} Such physiological cell death, in the absence of inflammation, is achieved by apoptosis, a structurally distinct programmed cell death pathway.\textsuperscript{19} Apoptosis is important in the regulation of normal cell population density, and may be one mechanism of deleting abnormal cells or cells that have been damaged by toxins, radiation injury, or other stimuli, and is thought to be responsible for numerous physiologic and pathologic event including the following: the programmed destruction of cells during embryogenesis and metamorphosis, cell deletion in proliferating cell population, cell death in tumors, death of immune cells, pathologic atrophy of hormone-dependent tissues, cell death induced by cytotoxic T-cells, cell death produced by a variety of injurious stimuli, etc.\textsuperscript{20} Apoptotic cell death can be distinguished from necrotic cell death. Necrotic cell death is a pathologic form of cell death resulting from acute cellular injury, which is typified by rapid cell swelling and lysis. In contrast, apoptotic cell death is characterized by controlled autodigestion of the cell.\textsuperscript{18} Apoptosis usually needs energy in the form of ATP. Calcium can activate latent enzymes that contribute to the structural change of apoptosis. Calcium-dependent proteases (eg, calpain) may also degrade the cytoskeleton. Apoptosis has distinct morphological features including compaction of chromatin against the nuclear membrane, cell shrinkage with preservation of organelles, detachment from surrounding cells, and nuclear and cytoplasmic budding to form membrane-bound fragments, known as apoptotic bodies, which are rapidly phagocytosed by adjacent parenchymal cells or macrophages.\textsuperscript{19} In contrast to necrosis, apoptosis does not provoke the release of cell content into the surrounding tissue of inflammatory reactions.\textsuperscript{20}

According to standard morphological descriptions, mitochondria were long thought to remain unchanged during apoptosis but to swell during necrosis.\textsuperscript{21} However, a review of the past literature on cell death, before apoptosis had even been described, reveals abnormal mitochondria in types of cell death that, retrospectively, can be classified as apoptosis. The most frequent abnormalities are a reduction in mitochondria size and a hyperdensity of their matrix, features often referred to as ‘mitochondrial pyknosis’.\textsuperscript{21,22,23} Namely, Following Bel-2-related proapoptotic protein Bax insertion into the outer mitochondrial membrane, mitochondria first condense (‘pyknotic mitochondria’), possibly fragment, and then cluster around the nucleus. The condensed mitochondria have lost their cytochrome C.\textsuperscript{24}

Fujiro S et al. demonstrated that apoptosis of neutrophils is modulated by psychophysical stress and its related hormones.\textsuperscript{25} Immobilization stress can enhance testicular germ cell apoptosis in rats.\textsuperscript{16} The effects of stress on immune responses have been attributed mainly to the adrenocortical hormones, glucocorticoids, which induce apoptosis in immunocompetent cells including thymocytes and peripheral T lymphocytes.\textsuperscript{26}

Recently, quite a few studies have shown that stresses are closely associated with diseases, especially with orofacial diseases. Black\textsuperscript{27} addressed that no disease is exempt from psychological influences, and, as a corollary, all diseases have psychological repercussions. Chun and Hong\textsuperscript{28} indicated that stress causes various forms of diseases in the orofacial area, including orofacial psychosomatic diseases in which emotional stress appears to play a major role
lichen planus, aphthous stomatitis), orofacial diseases in which psychologic factors appear to play a role (erythema multiforme, geographic tongue), orofacial infections where emotional stress is a significant predisposing factor (recurrent herpes labialis, acute necrotizing ulcerative gingivitis), orofacial lesions induced by neurotic habits inflicting trauma (biting of oral tissues, physical trauma with foreign objects, bruxism and clenching), neurotic orofacial symptoms (halitosis, xerostomia, burning mouth syndrome), and orofacial pain induced by emotional stress (temporomandibular disorder [TMD], atypical odontalgia).

Among the stress-related orofacial diseases, increasing attention has been recently addressed to TMD. Temporomandibular disorder (TMD) refers to pains and associated complaints involving the temporomandibular apparatus and the muscles of mastication. The TMD patient is likely to complain of tenderness on the TMJ and the masticatory muscles, limitation of the mandibular movement, joint sound, facial deformity and headache, earache, neckache and toothache, etc. The TMJ is the area where temporomandibular articulation occurs. It is certainly one of the most complex joints in the body, technically considered a ginglymoarthroidal joint which provides for hinging and gliding movements by the presence of the articular disc. Because it is also a synovial joint, it is governed by the same basic orthopedic principles that apply to other human synovial joints and subject to the similar pathologic disorders. Historically the field of TMD has concentrated on two theoretic approaches: the tooth–muscle theory (structure–function) and the psychophysiologic theory. Because a gold standard for clinical research and animal model are not available at present, the tooth–muscle theory has not been accepted. The psychophysiologic theory states that TMD is basically a psychosomatic and psychosocial disorder, that is a subdivision of a more deeply seated reaction to stress. Because of the intricacy of the neuronal connections of the teeth, mandible and muscles for mastication to the central nervous system and their close relationship to behavior, the explanation of these two theories represents an oversimplification of the cause of TMD. Recent perspectives on temporomandibular disorders have proposed that they are multifactorial problems with structural (occlusion), function (bruxism), external trauma, arthritic deterioration and psychological (anxiety, tension) factors as interrelated causes. Zhang ZK at al suggested that the four main contributing factors of TMD are microtrauma of TMJ, immune responses within TMJ, psychosocial factors, and anatomical structures of the TMJ itself. Possible mechanisms of the interactions of the four factors are presented, and principles of preventing and treating TMD are also suggested.

The psychosocial factors relate to causation, maintenance, or exacerbation of symptoms and furthermore, they make the treatment of chronic symptoms difficult and last other contributing factors. However, the pathologic mechanism of TMD induced by stress remains vague, so we have researched on it in this study.

The synovial membrane of the temporomandibular joint (TMJ) lines all the intraarticular structures except the articular cartilage of the articular eminence, fossa and mandibular condyle, and the articular disc. The synovial membrane is responsible for the production of synovial fluid, which is characterized by well-defined physical properties of viscosity, elasticity, and plasticity, and the removal of extraneous material shed into the joint cavity. The synovial membrane consists of two layers, a cellular intima resting on a vascular subintima, which in turn blends with the fibrous tissue of the capsule. The synovial intima cells can be differentiated into two main types on the basis of characteristic electron microscopic feature: Type A (macrophage–like) cell and Type B (fibroblast–like) cell. Type A cell are characterized by the present of prominent Golgi complexes and many related vesicles and vacuoles, whereas rough endoplasmic reticulum (RER) is relatively scanty. Type B cells are characterized by the present of
prominent RER, whereas only a few Golgi complexes are found.\textsuperscript{80}

Recent evidence suggests that the failure of cells to undergo apoptotic cell death might be involved in the pathogenesis of a variety of human diseases, including cancer, autoimmune diseases, and viral infections. In addition, a wide number of diseases characterized by cell loss, such as neurodegenerative disorders, AIDS (acquired immunodeficiency syndrome), and osteoporosis, may result from accelerated rates of physiologic cell death.\textsuperscript{18}

Apoptosis has been shown to be involved in remodeling of organ during development, and derangement of the apoptotic process may result in temporomandibular joint dysfunction or congenital malformation.\textsuperscript{38}

Observations on the synovial membrane from a TMD patient revealed degenerative changes including a reduction in macrophage-like synovial cells and abundant accumulation of dense fibrinlike material in the intercellular matrix, which interferes with the diffusion of nutrients from the underlying vascular channels to the joint cavity and thus results in damage to the cartilage.\textsuperscript{39,40}

Takahashi T et al\textsuperscript{41} suggested that levels of several proinflammatory cytokines is increased in synovial fluids of certain patients with TMD and these cytokines may play a role in the pathogenesis of synovitis and degenerative changes of the cartilagenous tissue and bone of the temporomandibular joint.

In rheumatoid arthritis (RA) synovial tissues, the synovial lining layer of the joint becomes thickened, hypercellular, and highly aggressive, and a paucity of apoptosis has been observed.\textsuperscript{42,43} If apoptosis would occur in the synovial cell layers, it may attribute to alteration of the viscosity of the synovial fluid and lead to impairment of lubrication and nutrition to the articular fibrocartilage and disc.\textsuperscript{44} In this present study, we proposed that stress may cause pathologic changes in the TMJ synovial membrane so lead to various TMD and other pathologic condition.

V. CONCLUSIONS

Stress and other psychological factors are believed to play an important role in the major health problems and also closely related with disorders and diseases of the orofacial tissue. Among the stress–related orofacial diseases, although increasing attention has been recently addressed to TMD, the pathologic mechanism of TMD induced by stress remains vague.

The present study was performed to observe the ultrastructural changes of TMJ synovial membrane of rats under restraint or cold stress in order to inquire the relationship between stress and pathologic changes of TMD.

Eighteen Sprague–Dawley rats (8-week-old, 323–367 g/bw) were used for the experiment and the rats were divided into 3 groups: 1) Normal control group; 2) Mild restraint stress group; 3) Cold stress group: the rats of restraint stress group were placed in the stress cage permitting slight movement throughout the period of experiment and the rats of cold stress group were immersed in cold water (4°C) for 6 min once a day throughout the experiment. All the animals were then sacrificed at day 1, 3, 5, and 7 day of the experiment and synovial membranes of TMJ were excised immediately and fixed in the glutaraldehyde in phosphate buffer. The synovial membranes samples were subjected to transmission electron microscopy.

The results were as follows:

Under the mild restraint stress

1. Mitochondria were partially condensed, and the number and the size of mitochondria were decreased gradually.
2. rERs were gradually dilated with irregular shape.
3. Double layer of nuclear envelope were slightly corrugated at day 7.

And under the cold stress

4. Mitochondria had shown slight swelling shapes, and were decreased in number at day 1, 3, but
again increased in number at day 5, 7, with very sound shapes.
5. The lamellated structures of the rER were changed to dilated pattern at day 1, 3, but were gradually recovered and activated at day 5, 7.
6. Nucleus was almost intact in all cold stress group during all period of the experiment.

So I can suggest that synovial membranes of rats may adapt to cold stress, a kind of physical stress, showing slight responses to stress. In contrast, cellular responses of the membranes to mild restraint stress which is known as a sort of psychological stress were increased as a result of failure in adaptation to the stress. It also appears that psychological stress is more potent than physical stress. Based on the observations, we hypothesize that if stress far exceeds the acceptable range for adaptation, apoptosis may occur in the TMJ synovial membrane and cause pathologic changes. Further studies need to be conducted to elucidate the pathologic mechanism.

REFERENCES

23. Hackenbrock, C.R. : Chemical and physical fixation of
33. (pain, 44(1991) 29–34 Psychological distress and diagnostic subgroups of temporomandibular disorder patients Charles P. McCready, Glenn T. Clark, Robert L. Merril, Virginia Flack and Mark E. Oakley

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Ultrastructural Changes on the Synovial Membrane of Rat Temporomandibular Joint under Mild Restraint and Cold Stresses
국문초록

한양, 중등도의 구속 스트레스시 용성백서의 측두하악관절 운활막의 미세구조 변화에 대한 전자현미경적 연구

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류종균·문경환·전양현·홍정표

오늘날 스트레스는 단순히 모든 현대인의 발상, 언론에 주도된 언론적, 경제적, 정치적, 사회적, 긴장적인 요인들로부터 받는 스트레스로 인한 결과로, 기능적, 형태학적, 생리학적, 신경계의 변화를 초래해 인체의 환경에 영향을 미치게 되는 것을 인식하고 있으며, 이에 대한 연구가 진행되고 있다. 이러한 결과의 발생과정에서 생체의 구조적, 기능적, 형태학적 변화가 초래될 때 apoptosis가 관여하고 있으며 이에 본 저자는 스트레스가 치과관절면의 성장이 있는 절병과의 상관관계를 규명하기 위해 이로의 한양 스트레스와 대조군의 균등한 중등도의 구속 스트레스를 부여한 후 측두하악관절 활막의 변화를 전자현미경으로 관찰하였다.

Sprague-Dawley 쥐를 용성백서(200-230 g/bw)의 33마리가 구속스트레스부여군(12마리), 한양스트레스부여군(12마리) 및 대조군(3마리)으로 나누고 이들을 각각 구속장치에 구속한 후 1, 3, 5일에 각각 희생시켰으며 측두하악관절 활막의 변화를 전자현미경으로 관찰하였다.

그 결과는 다음과 같다.

1. 중등도의 구속스트레스군에서 사립체는 부분적으로 농축된 소견을 보였으며 수와 크기에 있어 시간이 지남에 따라 점차 감소하는 소견을 보였다.
2. 중등도의 구속스트레스군에서 파리내혈관은 점점 확장되었으며 불규칙한 형태를 나타내었다.
3. 중등도의 구속스트레스군에서 물질보양의 혈액이 이중구조가 7일군에서 관찰되었다.
4. 한양 스트레스군에서 사립체는 1일, 3일군에서 약간 부분 형태를 가지고 그 수가 전소 감소되었으나 시간이 지남에 따라 5일, 7일군에서 경감한 모양으로 점차 증가되었다.
5. 한양 스트레스군에서 파리내혈관은 1일, 3일군에서 불규칙하게 확장된 소견을 보였으나 5일, 7일군에서는 잘 발달된 형태로 해 주위에서 다수 관찰되었다.
6. 한양 스트레스군에서 빈은 전 기간에 걸쳐 큰 변화를 보이지 않았다.

이와 같은 결과를 토대로 측두하악관절 활막조직은 한양 스트레스 및 중등도의 구속 스트레스에 대하여 중등도의 저항성을 가지고 있는 것으로 생각되며 생리적 작용관계를 넘어서 과도하고 지속적인 스트레스에 의하여 활막조직이 apoptosis되어 측두하악관절에 병리학적 변화를 초래할 가능성이 있다고 사료되어 이후 지속적인 연구가 요구된다.