Effect of Oriental Anti-Stress Agent(Bohyulanshintang) on the Salivary Gland of Rats under Restraint Stress

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CONTENTS

I. INTRODUCTION

It is no exaggeration to say that we are living in an era of 'stress.' The number of people complaining of fatigue, headache, sleep disorder, temporomandibular disorder and so forth is increasing with time. They are the common symptoms associated with stressful situations[1)-4)].

Selye applied the term 'stress' to the nonspecific biological response characterized by the activation of the pituitary adrenal axis and 'stressor' to all the stimuli that can induce stress. Animals subjected to prolonged stress conditions also presented a characteristic triad of structural changes including enlargement of the adrenal cor tex, atrophy of the thymus and the lymph nodes, and development of ulcers of the stomach and intestines[5)]. The theory of stress in oriental medicine is introduced from the concepts of wholism between 'nature and human,' 'parts and whole'and 'mind and body.' Stressor contains all pathological factors such as 'endogenous,' 'exogenous' and 'non endo-exogenous pathological factors'[6)].

It is an appealing notion that psychosocial factors can modulate the human response. Stress is increasingly reported to be associated with immunosuppression. The concept that psychic distress may predispose to medical illness is centuries old, but only recently has it attracted the attention of the scientific community at large[8)]. Psychoneuroimmunology has been established as a new field of mind-body connection, which has something to do with oriental medicine[6)].

Stress can induce apoptosis[9)-14)] which may be important in body defense mechanism. It is reported that psychophysical stress and its

related hormones modulate the apoptosis of the immune cells, suggesting that psychoneural systems may exert an effect on host defense[9].

Over the recent years cell death (apoptosis) has been extensively studied. Apoptosis is found to play a key role in proliferation, differentiation, embryogenesis, morphogenesis and homeostatic processes. Apoptosis abnormalities significantly contribute to such numerous human diseases as cancer, AIDS, degenerative pathologies of nervous system and developmental abnormalities. The elucidation of apoptosis mechanisms may promote our understanding of pathogenesis of various diseases and facilitate search for their treatment[15].

Recent research on causes of disease and aging has increasingly supported the importance of stress. One theory of the relationship between stress and disease is based on the concept of homeostasis. Homeostatic power of the body maintains the balanced, integrated condition we recognize as health. Failures in this capacity, such as those produced by frequent stressful experiences, can result in disease or death[16]. In oriental medicine, disease is thought to be originated from excess of pathological factors or insufficiency of living body's resistance. It is explained as the mutual action between the chunggi(正氣; healthy energy) and sagi(邪氣; evil factor)[6].

Clusterin is among the body defense mechanisms that can maintain the homeostasis of the body. Since it is expressed highly sensitive to environmental changes, it appear ubiquitously as a nonspecific cellular response to various insults like stress. Ample evidence suggest that clusterin has antiapoptic and cytoprotective functions, maintaining body homeostasis[19)-21)].

In order to maintain health and prevent disease, coping with various stress, very different interventions such as cognitive-behavioral therapies, techniques that elicit the relaxation response, meditation, exercise, increasing social networks, medicine and religion have been used[22,63)]. In oriental medicine, it appears that to cope the stress to maintain the homeostasis, herb medicine and Qigong have been accepted as effective ways to do so[6].

It is of a particular interest that whether herb medicine which has been used traditionally for relieving stress without significant side effect may protect the salivary glands from pathologic changes by stress.

Therefore, the present study was performed to observe the effect of Bohyulanshintang on the salivary glands under stress in association with expression of clusterin in the glands.

II. MATERIALS AND METHODS

Experimental animals and tissue preparation

Thirty three Sprague-Dawley rats (7-week-old) were purchased from Dae-Han Experimental Animal Research Center, Seoul, Korea. They were maintained at 20-23℃ and fed *ad libitum* on a normal laboratory diet. The rats were divided into 3 groups: Group I, 3 normal control rats; Group II, 15 rats were placed in the stress cages; Group III, 15 rats were administrated Bohyulanshintang (1.9g/kg/day), p.o., for 10 days before they were placed under the restraint stress. All the animals were then sacrificed at day 0, 1, 3, 5, 7 day of the experiment and the submandibular glands were excised immediately. After fixation with Bouin solution overnight, the tissues were embedded in paraffin resin. Serial paraffin sections were made (5㎛), placed on poly-L-lysine coated slides, and stored at -70℃ until use.

Preparation of clusterin antibody

Prior to raise antibody against clusterin, a synthetic peptide corresponding to the sequence of 144-158 amino acids (NH_2-GDRIDSLMEN-DRQQS-COOH;1865.9 atomic mass units) from the porcine clusterin α-subunit was prepared by Fmoc peptide synthesis procedure and purified by repeated HPLC. The α-peptide (2.2mg) was conjugated to 2mg of cathionized BSA using SuperCarrier EDC system(Pierce Rockford, IL, USA). A New Zealand white rabbit was injected at multiple sites subcutaneously with the conjugated peptide-carrier in complete Freund's adjuvant. Starting one week after the first injection, the rabbit was boosted weekly for 2 weeks with the same conjugate in incomplete Freund's adjuvant. Ten days after the last third injection, the rabbit was bled and antiserum was collected.

Immunohistochemistry

Immunohistochemical analysis was performed on the paraffin sections by avidin-biotin-peroxidase complex (ABC) method as described previously using a specific antibody against clusterin α-peptide[23]. Briefly, the sections were deparaffinized in xylene, hydrated, washed in phosphate buffered saline (PBS), and incubated twice in methanol containing 0.5% H_2O_2 for 5 min. each at room temperature. After rinsing with PBS, the slides were incubated in PBS containing 0.1% Triton X-100 for 10 min, and then immersed in normal goat serum diluted with rabbit IgG anti-clusterin α-peptide for 24 hr at 4℃. After washing with PBS, the slides were incubated with biotinylated goat anti-rabbit immunoglobulin for 1 hr at room temperature. The slides were then washed in PBS and incubated with avidin-biotin-peroxidase complex (Vector Lab., USA) for 1 hr. Thereafter, immunohistochemical reactions were detected by coloration using diaminobenzidine solution (100mM Tris, pH 7.4, 0.01% H_2O_2, 0.05% diaminobenzidine hydrochloride).

Northern blot analysis

Total RNA was isolated from the submandibular glands by the acid guanidium thiocyanate and phenol/chloroform extraction method as described by Chomezynski and Sachci[24]. The RNA (10μg) was denatured and fractionated in a 1.2% agarose gel containing 0.67M formaldehyde, and then transferred to positively charged Hybond-N^+ nylon membrane (Amersham, Arlington Heights, IL, USA) by capillary action with 10×SSC. The membranes were baked at 120℃ for 30 min and prehybridized in hybridization buffer (50% formamide, 1.5×SSC, 5×Denhardt's reagent, 1.0% SDS, 20mM sodium phosphate, pH 7.0, and 100μg/ml yeast RNA) at 55℃ for 2 hr. T7 RNA polymerase was used to generate [α-^{32}P] UTP-labeled cRNA antisense probe (2×106 c.p.m./ml). After hybridization with the probe at 55℃ for 16 hr, the membranes were washed twice in 0.5×SSC/0.1% SDS at room temperature for 16 min each, and more stringently once in 0.2×SSC/0.1% SDS at 65℃ for 30-45 min. The membranes were then exposed to X-ray film with an intensifying screen at -80℃ for 2 days.

Ⅲ. RESULTS

Immunohistochemistry of clusterin protein in the submandibular glands

To localize the expression of clusterin protein in the submandibular glands, the tissues of each

group were fixed and processed for immunohistochemistry using the specific antibody against clusterin α-subunit peptide.

In the normal control group, clusterin immunoreactivity was detected slightly in the ductal cells(Fig. 1).

In the restraint stress group, clusterin was observed immediately after the application of the stress and then disappeared. However, the clusterin expression was found to be weak. By the restraint stress, histologically, apoptosis was observed, showing karyorrhectic and pyknotic changes. Besides, acinic cells were destructed earlier than ductal cells(Fig. 2-4).

In the Bohyulanshintang-administrated and restraint stress groups, it was found that expression of clusterin in the submandibular glands was increased with time until day 5 of the experiment. But at day 7 of the experiment, the expression of clusterin was weakly observed. None of rats showed a sign of apoptosis through the experiment(Fig. 6-10).

Northern blot analysis of clusterin mRNA in the submandibular glands

Northern blot analysis of clusterin mRNA isolated from the submandibular glands showed a band of approximately 2.0kb in size and the equality of RNA loading was demonstrated by the ethidium bromide staining of 18S rRNA.

In the restraint stress group, mRNA of clusterin was detected only at the day of the application of stress, although the level of the expression was very low (Fig. 5).

In the Bohyulanshintang-administrated and restraint stress group, mRNA of clusterin was mildly expressed through all days of the experiment (Fig. 11).

IV. DISCUSSION

Stress is the nonspecific response of the body to any demand[5] and has been believed to be the cause of various forms of diseases for a long time[25].

Life and health in a modern industrial society require a continuous adaptation of the body to a variety of extraneous influences. Adequate adaptation is brought about by a balanced interaction between the nervous system, the endocrine organs and the immunocompetent tissues[26].

Recent research on causes of disease and aging has increasingly supported the importance of stress. One theory of the relationship between stress and disease is based on the concept of homeostasis[15]. Besides disease can be regarded as a phenomenon that occurs when an agent or condition threatens to destroy the dynamic steady state upon which the integrity of the organism depends[27].

It is conceivable that apoptosis induced by disturbed homeostasis is one of the causes of human diseases such as cancer, infectious diseases, barrier dysfunction and aging etc[7-9,28,29,62].

Apoptosis is a process of genetically programmed alterations of cell structure that lead to failure of proliferation and differentiation, and eventual cell death[10]. Apoptosis (programmed cell death) and necrosis can be readily distinguished morphologically and biochemically. The chief morphologic features of apoptosis are cell shrinkage, chromatin condensation, formation of apoptotic bodies and phagocytosis of them by adjacent healthy cells[30]. This morphologic pattern of cell death should be differentiated from the necrosis, the more common type of cell death after exogenous stimuli, occurring after hypoxia and chemical

injury, and manifested by severe cell swelling or cell rupture, denaturation and coagulation of cytoplasmic proteins, and breakdown of cell organelles[30]. The most striking biochemical change observed in apoptotic cells is the cleavage of the genomic DNA into discrete nucleosome-sized fragments, producing a laddering pattern when the DNA is examined electrophoretically[29].

It is reported that stress-induced apoptosis may occur by high glucose, increased glucocorticoid and direct nerve involvement. With this, we should consider such basic psychoneuroimmunologic concept as followings.

The activation of CNS by psychosocial factors, in particular by stressors develops into alterations in the immunocompetence or anatomicofunctional barriers of the host or in microbic virulence along two different pathways: biological/direct and behavioral/indirect. The principal mechanisms of the biological/direct pathway include the hypothalamic-pituitary-adrenal (HPA) axis and of the sympathetic nervous system (SNS). Coticosteroids and cathecholamines are the two principle mediators of neuroimmunomodulation[7].

With regard to glucose, epinephrine is a cathecholamine released from the adrenal glands in response to neural signals that trigger the "fight-or-flight" response. Among its diverse physiological effects, epinephrine stimulates increased breakdown of glycogen, resulting in elevated intracellular levels of glucose 6-phosphate. This increase in glucose 6-phosphate leads to enhanced glycolysis in muscle and to an increase in glucose released to the blood stream from liver[31]. In contrast glucocorticoids have the ability to stimulate gluconeogenesis by the liver and cause a moderate decrease in the rate of glucose utilization by the cells everywhere in the body, which results in elevated blood glucose concentration[32]. High ambient glucose affects endothelial and other vascular cells at the cellular level, delays endothelial cell replication by retardation during S-phase in various species and causes excessive cell death[33]. High concentrations of glucose are considered to be toxic for the pancreatic β-cell[34]. And it is reported that apoptotic cellular changes can be prevented in vivo by treating hyperglycemic mice with insulin before and immediately after conception[35]. In addition, poor utilization of social support is associated with the onset of glucose tolerance abnormality[36].

In case of glucocorticoids, principal effectors in the stress response, they have profound effects on mood and behavior and affect neurochemical transmission and neuroendocrine control. Recently the role of glucocorticoids in stress-related neuronal plasty is suggested[14]. Concordet et al. reported that physical stress induces glucocorticoid receptor-mediated apoptosis of rat thymocytes[13].

Researchers have strongly hoped to demonstrate the existence of specific pathways in which immunocytes can be directly regulated. Saito [37] showed by electron microscopic observation how the immunocytes in the guinea pig spleen are directly innervated.

Besides, inappropriate cell-matrix interaction results in apoptosis, which may account for cell death mechanisms during developmental processes or under pathological conditions[38]. Growth factor deprivation and ionizing radiation are related to apoptosis[10]. Simultaneous induction of a heat shock response and an oxidative stress response is responsible for human endothelial cell apoptosis[11].

Clusterin is an intriguing, ubiquitous, and highly conserved glycoprotein. It is secreted as a heterodimer of 70~80kDa glycoprotein,

comprising α and β subunits[39,40]. It has potential amphipathic helical domains that allow this protein to bind to hydrophilic molecules, as well as potential heparin-binding domains responsible for interaction with the cell membranes and extracellular matrices[41].

Clusterin is a widely expressed, well conserved, secreted glycoprotein, which is highly induced in tissues regressing as a consequence of apoptotic cell death. Clusterin expression is only confined to surviving cells following the induction of apoptosis[42] as an extracellular version of heat-shock protein[44]. Clusterin synthesized by apoptotic cells can be immunologically distinguished from clusterin synthesized by surviving cells in damaged tissue[43]. Pearse et al.[61] reprted that clusterin is not critical to the mechanism of cell death although it is the first example of a readily detectable marker which is differentially expressed in cells undergoing apoptosis, emphasizing that apoptosis is not a uniform phenomenon, but is dependent on the nature of the cells involved and the means of induction.

The cytoprotective mechanism of clusterin has been proposed based on the findings that it inhibits complement-mediated lysis, binds to surface active toxic hydrophobic compounds and neutralizes at or near the cell membranes by formation of soluble complex, and preserves the integrity of the barrier as a potent cell aggregation and adhesion molecule[20,21].

As stated above, stress is important in the pathogenesis including apoptosis and expression of clusterin which protects the cells from the stress in the view of psychoneuroimmunology. This is the case in the orofacial region.

The relationship of stress to the orofacial area can be considered in four aspects[25]: normal physiological and psychological functions of the mouth, stress-relieving orofacial activities, stress and dental treatment, and stress-related orofacial disorders and diseases.

Recently a few studies have suggested that stress is strongly associated with orofacial diseases. Chun and Hong[45] indicated that stress causes various forms of diseases in the region 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, benign mucous membrane pemphigoid, 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, leukoplakia due to smoking, bruxism and clenching), neurotic orofacial symptoms (xerostomia, halitosis, burning mouth syndrome, altered or loss of taste perception, pain or discomfort with no tissue change), and orofacial pain induced by emotional stress (temporomandibular disorders, muscle tension headache, atypical odontalgia).

In order to prevent and treat the stress-related symptoms and disease in dental or medical field, stress-blocking or preventing methods are necessary. Very different techniques including cognitive or behavioral psychotherapy, hypnosis medicine, meditation, exercise, increasing social networks, and religion have been used to cope with stress[22,63-66].

Some traditional oriental medicines have been proved to be effective in preventing apoptosis. It is reported that triptolide induces T cell apoptosis through activating caspases[46], the water fraction of ginseng can exert a potent effect on the recovery of the hair follicles by its combined effects on proliferation and apoptosis of the cells

in the hair follicle[47] and various herbal medicines have inhibitory effects on glucocorticoid-induced apoptosis in thymocytes[48].

Bohyulanshintang believed to protect the body from interaction between the chunggi(正氣; healthy energy) and sagi(邪氣;evil factor) has been widely described against stress[6]. It is composed of Discoreae Radix, Angelicae gigantis Radix, Atractylodis Macrocephalae Rhizoma, Raphani Semen, Louganae Arillus, Rehmanniae Radix, Liriopis Tuber, Poria (Hoelen), Zizyphi Semen, Paeoniae Radix, Massa Medicata Fermentata, Hordei Fructus Germinatus, Cnidii Ruizoma, Polygalae Radix, Maximowicziae Fructus, Scutellariae Radix, Glycyrrhizae Radix, Amoni Semen, Chrysanthemi Flos[49-54]. And it is proved that Bohyulanshintang has an effect on stress-related disease by the experimental study on catecholamine concentration in urine[49], behavior modification[50], change of weight and blood serum[51], gastric ulcer, catecholamine concentration in blood[52], catecholamine content of brain and its each part[53,54].

It has been reported that morphology and function of the salivary glands are vulnerable to stress : the inhibition of salivary gland function is due to central influences from higher centers acting on the salivary centers and thereby suppressing reflex activity[55]; the submandibular gland. is more sensitive to cold than the sublingual gland[56]; repeated stress, even of temporary duration, is able to influence directly or indirectly the morphofunctional state of the submandibular gland[57]; acute emotional painful stress enhances free-radical lipid oxidation in the salivary gland tissues and reduces the activities of antioxidant enzymes in it[58]. In addition, submandibular gland is reportedly more age-related, in the pattern of fluid secretion with an intense gustatory stimulus than parotid gland[59].

Hong et al.[60] reported that clusterin is expressed in the salivary gland of the rat in the early stage of cold stress but disappeared since then. They indicated that clusterin would not be expressed if the cold stress is continued, since no need of clusterin production is required by the cells which are already adapted to the stress and recovered from the stressful condition.

In the present study, we examined the expression of clusterin in the salivary glands, the orofacial tissue-fluid interface, under restraint stress condition with and without administration of Bohyulanshintang by immunohistochemistry and Northern blot analyses, in order to inquire the relationship between stress and orofacial diseases, and to examine the effectiveness of anti-stress effect of Bohyulanshintang on the salivary glands.

In the restraint stress group, it was found that clusterin was expressed in the submandibular glands immediately after the application of the stress. However the expression of clusterin was weak. It was also observed that the glandular cells were apparently apoptotic.

In the Bohyulanshintang-administrated and restraint stress group, the expression of clusterin in the submandibular glands were gradually increased with time until day 5 of the experiment. At day 7 of the experiment, clusterin was weakly observed. No apoptotic cell was found through the period of the experiment in the group.

The overall results suggest that as a response to stress, clusterin is expressed in the glands to protect the glandular cells from the stress. But if stress is so strong and prolonged that it can exceed the stress adaptability of the cells, then the cells may undergo apoptosis instead of producing clusterin. It is conceivable that Bohyulanshintang may promote the adaptability

of the glandular cells to stress so that apoptosis of the cells may not occur.

In summary, stress may cause apoptosis depending upon degree, duration of the stress applied and host's adaptability. Clusterin can be expressed as a stress protein to minimize cell damage and ensure cell viability, probably, only if the stress falls within the bearable range for the cells. If the stress far exceed the range, this is not the case. A strong stress may suppress the production of clusterin by the cells but induce apoptosis of the cell. Since stress-induced apoptosis alters the normal salivary physiology, we propose that pathologic change of the salivary glands can be induced by stress and subsequently by apoptosis. It is also proposed that oriental anti-stress medicine like Bohyulanshintang can more or less prevent the stress-related pathologic changes of the body and may be clinically used with minimal side effect.

V. CONCLUSIONS

Recent studies on the causes of disease and aging has increasingly supported the importance of stress. Despite evidence suggests that orofacial diseases are also closely associated with stress, the pathology remains vague. Although a variety anti-stress drugs have been developed, their clinical use is limited because of their side effect. In oriental medicine, some herb prescriptions have been traditionally used to relieve stress without having any significant side effect. Effect of Bohyulanshintang on the salivary glands and the expression of clusterin under restraint stress was observed in order to check whether the medicine can be used for prevention and treatment of diseases of salivary glands which are vulnerable to stress.

Thirty three Sprague-Dawley rats were divided into 3 groups: Group I, 3 normal control rats; Group II, 15 rats were placed in the stress cages; Group III, 15 rats were Bohyulanshintang administrated(1.9g/kg/day), p.o., for 10 days before they were placed under the restraint stress. All the animals were sacrificed at day 0, 1, 3, 5, 7 day of the experiment and the submandibular glands were excised immediately. The levels of clusterin proteins and mRNA in the tissues were measured by immunohistochemistry and Northern blot analyses, respectively. The results were as follows:

1. In the restraint stress group, immunohistochemistry and Northern blot demonstrated that clusterin was expressed only immediately after the application of restraint stress and apoptosis was induced with karyorrhectic and pyknotic changes at day 3 and 5.
2. In the Bohyulanshintang-administrated and restraint stress group, the expression of clusterin in the submandibular glands is gradually increased with time until day 5 of the experiment. At day 7 , the expression of clusterin was found to be decreased.
3. In the Bohyulanshintang-administrated and restraint stress group, no sign of apoptosis was observed throughout the period of the experiment.
4. In the Bohyulanshintang-administrated and restraint stress group, Northern blot showed that mRNA of clusterin was weakly expressed through all days of the experiment.

The overall results suggest that as a response to stress, clusterin is expressed in the glands to protect the glandular cells from the stress. But if stress is so strong and prolonged that it can exceed the stress adaptability of the cells, then the cells may undergo apoptosis instead of producing clusterin. It is conceivable that

Bohyulanshintang may promote the adaptability of the glandular cells to stress so that apoptosis of the cells may not occur.

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국문초록

補血安神湯이 스트레스에 의한 백서 타액선 조직 변화에 미치는 영향에 관한 연구

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일상 생활에서 우리는 스트레스에 항상 노출되어 있으며, 스트레스는 생체의 신경계, 내분비계 및 면역계의 변화를 수반한 항상성의 파괴로 수많은 정신적, 육체적 질병을 야기시킨다. 특히 구강안면영역에서도 다양한 구강점막질환과 구강건조증 등을 발생시킨다.

스트레스를 제거하는 방법으로는 약물요법 및 상담, 명상요법, 종교요법 등 다양한 방법이 제시되고 있는데, 다소의 부작용이 나타나거나 꾸준히 시행하기가 쉽지 않으며 스트레스의 원인을 근본적으로 제거하기가 현실적으로 용이하지 않은 경우가 많아 스트레스에 대한 해결책에 대하여 많은 관심이 집중되고 있다.

이에 본인은 스트레스가 가해졌을 때 백서 악하선에서 관찰되며 apoptosis에 대하여 세포보호작용을 하는 clusterin(SGP-2)을 이용하여 구속스트레스를 가하기에 앞서 오랫동안 경험적으로 사용되어 왔고 부작용이 적은 전통약물인 보혈안신탕을 투여하고 스트레스에 의한 타액선의 조직변화를 관찰하여 그 효과를 확인해 보고자 하였다.

Sprague-Dawley계 웅성 백서(200-230g/bw) 33마리를 정상 대조군(3마리), 구속스트레스군(15마리) 및 보혈안신탕 투여 후 구속스트레스군(15마리)으로 나누고 이들을 각각 구속장치에 구속한 후 0, 1, 3, 5, 7일에 희생시켜 악하선을 적출하였으며, 면역조직화학법 및 Northern Blot을 이용하여 clusterin의 변화를 관찰하였다.

그 결과는 다음과 같다.

1. 구속스트레스군의 악하선 조직에서 clusterin 단백질과 mRNA는 실험 즉일군에서만 미약하게 관찰되었으며 실험 3일과 5일 후에 핵붕괴 및 핵농축 등의 핵변화를 동반한 apoptosis가 관찰 되었다.
2. 보혈안신탕 투여 후 구속스트레스군의 악하선 조직에서 실험 5일군까지 clusterin 이 증가한 후 실험 7일군에서는 감소하였다.
3. 보혈안신탕 투여 후 구속스트레스군의 악하선 조직에서는 apoptosis가 관찰되지 않았다.
4. 보혈안신탕 투여 후 구속스트레스군의 악하선 조직에서 clusterin mRNA가 실험 전군에 걸쳐 미약하게 관찰되었다.

이상의 결과로 타액선 조직은 스트레스 단백질인 clusterin을 생산하여 세포를 보호함으로써 스트레스 상황에 적응하지만, 생리적 적응한계를 넘는 스트레스에 노출될 때는 apoptosis됨이 확인되었다. 그리고 보혈안신탕은 스트레스 상황에서 세포의 생리적 적응력을 높여 세포의 apoptosis를 억제하는 효과를 나타냄이 확인되었다.

따라서 본 연구결과는 구강건조증등의 스트레스성 타액선 질환의 병리기전을 규명하는데 도움이되리라 생각되며, 향후 항스트레스 효과를 가진 보혈안신탕등의 한약재를 임상에 적용함으로써 스트레스로 인한 신체의 병리적 변화를 다소나마 차단할 수 있을 것으로 사료된다.

Explanation of Figures

Fig. 1 : Immunolocalization of clusterin protein in the normal submandibular gland.
Fig. 2 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress immediately after the application of the stress.
Fig. 3 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress at day 3 of the experiment.
Fig. 4 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress at day 5 of the experiment.
Fig 5 : Northern blot analysis of clusterin mRNA in the submandibular gland under restraint stress.
Fig. 6 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress with Bohyulanshitang immediately after the application of stress.
Fig. 7 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress with Bohyulanshitang at day 1 of the experiment.
Fig. 8 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress with Bohyulanshitang at day 3 of the experiment.
Fig. 9 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress with Bohyulanshitang at day 5 of the experiment.
Fig 10 : Immunolocalization of clusterin protein in the submandibular gland under restraint stress with Bohyulanshitang at day 7 of the experiment.
Fig.11 : Northern blot analysis of clusterin mRNA in the submandibular gland under restraint stress with Bohyulanshitang.

사진부도

Fig. 1

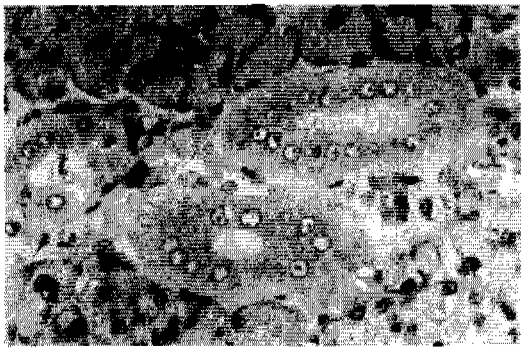


Fig. 2

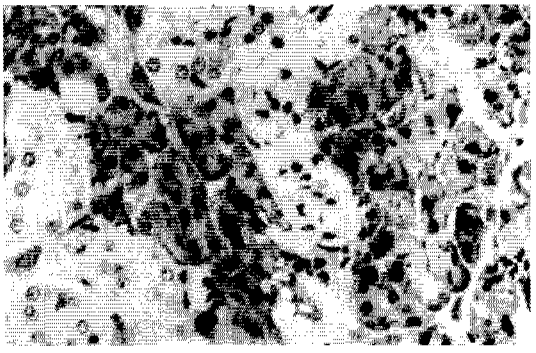
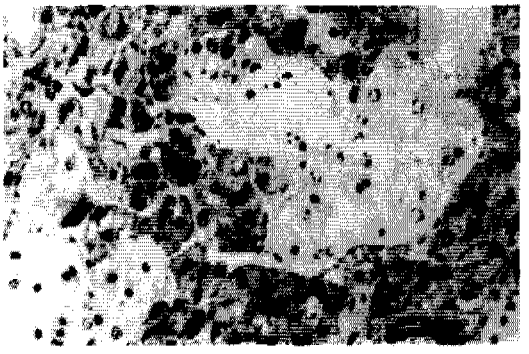


Fig. 3



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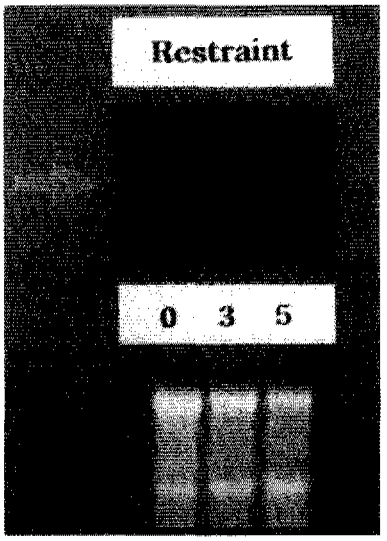
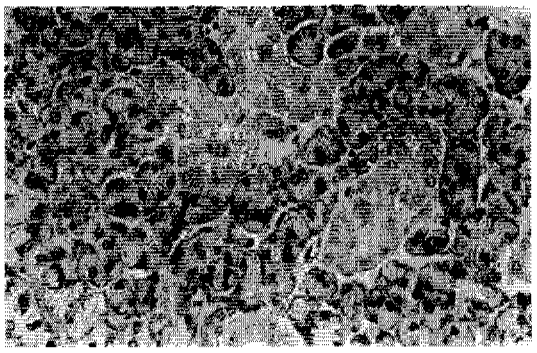


Fig. 5



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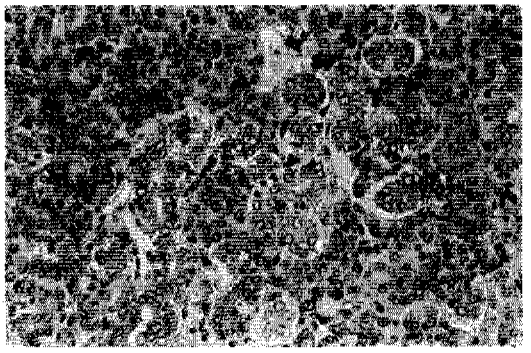

Fig. 7

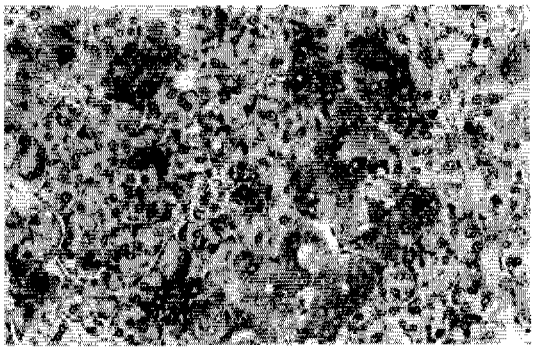

Fig. 8

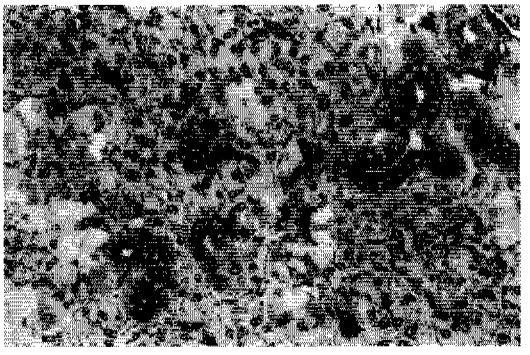

Fig. 9

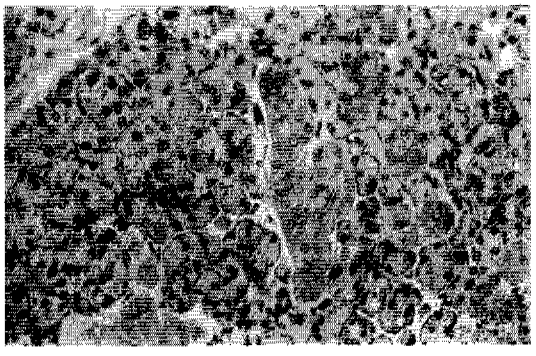

Fig. 10

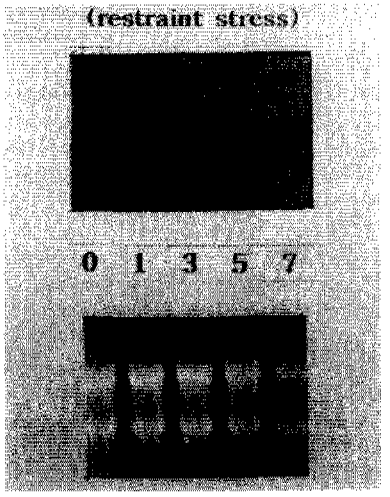

Fig. 11