

## IMMUNOHISTOCHEMICAL EXPRESSION OF HEAT SHOCK PROTEIN27 IN THE MOUSE DENTAL PULP AFTER IMMEDIATE TEETH SEPARATION

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### Abstract

*Aim:* After immediate teeth separation, expression of HSP27 in the mouse dental pulp was examined. Immunohistochemistry was performed to examine the incidence of HSP27 expression.

*Materials and methods:* A total of 36 8-week-old ddY mice were used as experimental subjects and a wedge was inserted in between maxillary right molars. The wedge was removed 30 min or 3 h after insertion. Animals were immediately sacrificed after the removal of wedge or until 1 week later and serial sections from paraffin-embedded tissues were prepared. Immunohistochemistry was carried out to examine the expression of HSP27. The untreated side served as the control.

*Results:* In the control group, the endothelial cells and some pulp fibroblasts weakly expressed HSP27 suggesting that the expression is due to mechanical stress brought about by physiological masticatory force and pressure from the tongue. In both 30 min and 3 h experimental groups, HSP27 expression was highest at 24 h after wedge removal and the expression remained the same or started to decrease thereafter. The expression decreased at the same level as that of the control group 1 week after wedge removal.

*Conclusion:* HSP27 may serve as an indicator of stimulus strong enough to show its expression.

*Key words:* Heat shock protein27, Mechanical stress, Immediate teeth separation, Dental pulp

### INTRODUCTION

During conservative dental treatment and restoration of contact area using composite filling, immediate teeth separation may be necessary by using a wedge or sometimes a separator. Even during food impaction, teeth are separated inducing mechanical stress. Stress does not directly act as pressure or traction on periodontal ligament. Mechanical stress affects the pulp and travels to the apical part going to the lateral branch and then to the periodontal tissues. In recent years, HSP25 expression has been reported in dental pulp after injury induced by cavity preparation and tooth replantation [1].

Previously, a cytotoxic protein known as heat shock protein (HSP) has been reported to occur as a major protein observed in various tissues and organs due to mechanical irritation or stress [2]. HSP is not only induced by heat shock but also by ischemia, various pathological changes like inflammation and infection; physical stress such as radiation, light, enzymes, ions; heavy metals like arsenic, arsenite, chemicals and variety of amino acid derivatives like ethanol and reactive oxygen species [3, 4]. HSPs have been associated in cell damage, defense and repair and are thought to be involved in controlling cell function [5-7].

HSP27 has role in suppression of apoptosis, control of protein expression, chaperone activity by binding to nascent protein, cell development and cell differentiation. It is also involved in cell signal transduction [8-12]. However, the dynamics and its role in pulp tissue in regulating cellular response to cytotoxic stimuli during experimental teeth separation have not been investigated. Shigehara et al. [13] reported HSP70 mRNA expression in dental pulp after orthodontic teeth movement and mentioned that mechanical stress is one of the factors in the expression of HSP70. Muraoka et al. [14] histologically observed HSP27 expression in pulp tissue and mentioned that biomechanical stress in periodontal ligament induced the expression and incidence of HSP27. The changes in the expression of HSP27 in the pulp in the control group were thought to be due to mechanical stress by orthodontic force. Thus, similar expression changes in HSP27 were observed in this research when teeth were separated using a wedge.

In this study, a detailed pathological investigation focused on the immunohistochemical expression of HSP27 in pulp cells after applying mechanical stress caused by teeth separation on experimental laboratory animals.

### MATERIALS AND METHODS

#### 1) EXPERIMENTAL ANIMALS

A total of 36 8-week-old ddY mice weighing  $35 \pm 5$ g (30-40g) were purchased from Japan SLC Inc (Hamamatsu, Japan). The mice were kept in an air-condi-



In 3 h group, HSP27 expression in the cytoplasm of endothelial cells became stronger (Fig. 3-b). In 9 h group, HSP27 became stronger and is expressed in the cytoplasm of endothelial cells as well as in some fibroblasts in the coronal pulp. In 24 h group, HSP27 expression remained the same with 9 h group (Fig. 3-c). In 3 days group, HSP27 expression in the odontoblast layer can still be observed but a decrease in expression is evident. In 1 week group, HSP27 further decreased with no difference with the control group (Fig. 4d).

#### *Experimental 3 h group* (Figs. 4, a-d; 5, a-d)

Immediately after removing the wedge, HSP27 was expressed by capillary endothelial cells surrounding the pulp and fibroblasts in the pulp (Fig. 4-a). In 3 h group, HSP27 was strongly expressed in the odontoblast layer (Fig. 5-a, b), cytoplasm of capillary endothelial cells surrounding the resident pulp cells (Fig. 5-c), cytoplasm of pulp fibroblasts (Fig. 5-d) throughout the pulp (Fig. 5-b; 5-d). In 9 h group, expression was stronger in the odontoblast layer, capillary endothelial cells of resident pulp cells and pulp fibroblasts. In 24 h group, HSP27 expression increased in the odontoblast layer, capillary endothelial cells of resident pulp cells, pulp fibroblasts (Fig. 4-c). In 3 days group, HSP27 can still be uniformly observed in the odontoblast layer, capillary endothelial cells of resident pulp cells, pulp fibroblasts but had weakened considerably. In 1 week group, HSP27 in the odontoblast layer, capillary endothelial cells of resident pulp cells, pulp fibroblast remained weak (Fig. 4-d).

## DISCUSSION

Qualitative observation on immunohistochemical expression of HSP27 using mice as experimental subjects was performed in this study. The number of animals used is large enough so that the difference in immune response will not be due to individual differences. Two groups were made and further divided into 6 groups consisting of 3 mice per group for a total of 36 mice.

Base on preliminary results, the buccal root of the right maxillary first molar would be the most appropriate area for observation. This is because when the coronal pulp and radicular pulp showed the same HSP27 staining condition after subjecting to mechanical stress observed in longitudinal section. Thus, it seemed to be the most affected compared to the other 2 roots (Fig. 2).

In addition, the 30 min separation time is the maximum separation time use in dental clinical settings. Also, each group provided 3 hours of time and about 6 times more to induce a more severe stress. The specimens were prepared immediately after the shortest separation time or from 3 h to 1 week to determine the prevalence of stress after removal of the wedge.

Separation of teeth during dental treatment is converted to mechanical stress that directly affects the periodontal tissues [14]. It can also be understood that the damage can act as a stimulus to the dental pulp in the same manner. In other words, some cytotoxic substances may cause nerve irritation through the vessel

relative to the movement in the apical foramen. The stimulus is thought to also take place in the lateral branch. However, in our search for literatures, no study has been conducted so far examining the expression of HSPs during cytotoxic changes in pulp cells. Sens et al. [15] reported the expression of HSP in odontoblasts, odontoblast's processes (dentinal fibers), dental pulp fibroblasts, endothelial cells, and vascular smooth muscle cells in human 3rd molars. Further, Tate et al. [16] and Suzuki et al. [17] verified the expression of HSP in dental pulp cells after experimental cavity preparation by laser ablation. Moreover, Matsuzaki et al. [18] reported an increased in mRNA levels of HSP27 in dental pulp cells after teeth separation has not been investigated. However, HSP27 expression has been implicated in the expression of Runx2 in dental pulp under mechanical stress in orthodontic researches [19, 20].

Recently, it is well known that homeostasis and expression of HSP27 can be stimulated by various injuries. HSPs are protein acquired for survival under harsh environment in the cell induced by non-physiological stimuli, as molecular chaperone [21] with anti-apoptotic feature which allows cell to escape from death [6, 7, 11]. In addition, many cells in response to stress and inhibit protein denaturation by repairing denatured proteins express HSPs. Previous in vitro and in vivo experiments found that HSP is a protein widely distributed in intracellular equilibrium, constitutively expressed under stress condition and actually even in unstressed condition, cell differentiation, proliferation, survival and essential various cellular functions such as maintenance of protein [7, 21].

HSPs like HSP70 and HSP90 are high molecular weight HSP temporarily attached to the immature state of protein known to act as a molecular chaperone with the ability to assist and mature protein folding and to mediate the meeting of polypeptides [22]. The speculation that low molecular weight HSPs function as molecular chaperone in cells is not clear [23].

This investigation on HSP27 was initially discovered as an inhibitor of actin polymerization [24]. Thereafter, high levels of HSP27 became known to be present at high levels in vascular smooth muscle cells in unstimulated conditions. From this, HSP27 is believed to have some physiologic role in vascular cells [25].

First, the dental pulp of the control group has always shown weak positive reaction to HSP27. Similar results to Nakano et al. [20] have been observed and it is presumed that the response is due to the load from physiological and biomechanical masticatory force and tongue pressure. In addition, HSP27 observed in the control group in pulp fibroblasts is weak and is mainly expressed by vascular endothelial cells. This expression was noted early during the experiment indicating that HSP27 plays a physiological role in vascular system [25]. In the experimental group with 30 min separation of teeth, strong HSP27 expression was observed in vascular endothelial cells as well as in some pulp fibroblasts. Also, this expression reached its maximum after 24 h and then gradually decreased until the expression is the same as the control group. This is due to the stress from separation of teeth,

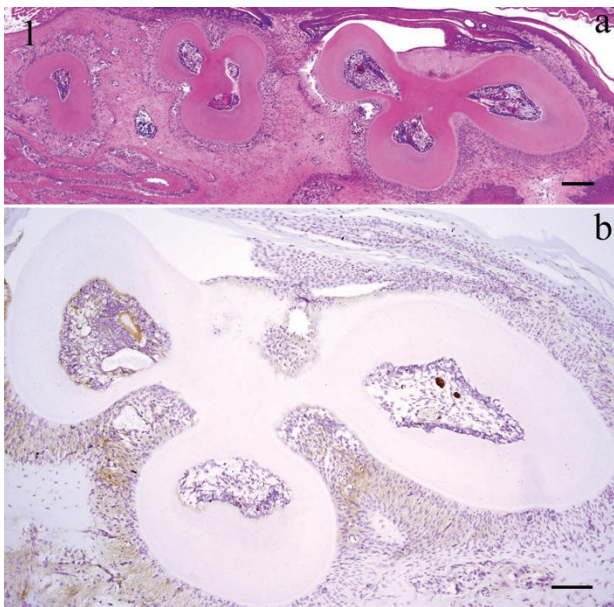


Fig. 1. Histopathological view of the horizontal section of a sample from the experimental group (a: Bar 200µm), immunohistochemical staining profile of HSP27 in the 1st molar, experimental group (b: 3h-3h, Bar 100µm).

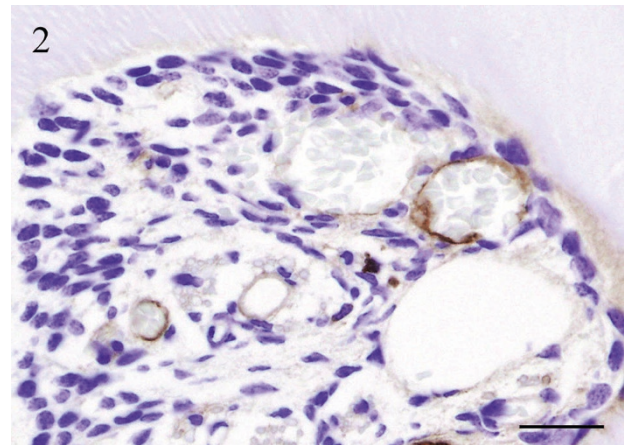


Fig. 2. HSP27 positive staining appeared in some endothelial cells (control, Bar 20µm).

Fig. 4. The positive reactions appeared in almost all dental pulp tissue cells (a: 3h-0m, Bar 20µm); staining reaction become strong (b: 3h-3h, Bar 20µm), the strong positive reactions are observed in pulp cells (c: 3h-24h, Bar 20µm), strong positive reaction in the pulp cells (d: 3h-1w, Bar 20µm).

Fig. 5. Immunohistochemical staining in the odontoblasts layers, positively stained small vessels are noted (a, b: 3h-3h, Bar 10µm). The experimental dental pulp shows strong positive reactions in endothelial cells (c: 3h-9h, Bar 10µm), weak positive staining in some dental pulp fibroblasts (d: 3h-9h, Bar 10µm) are noted in the pulp chamber.

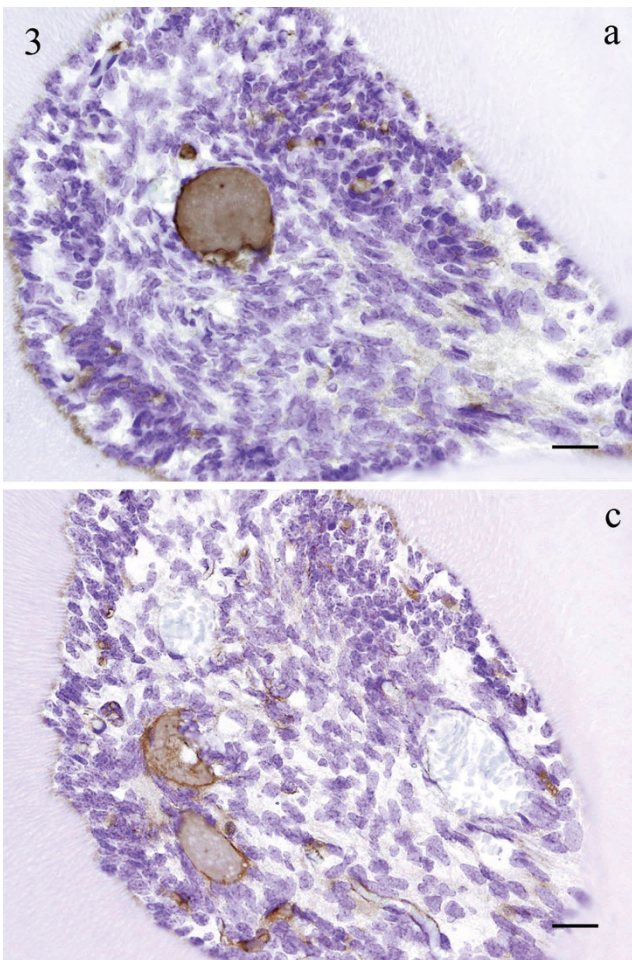
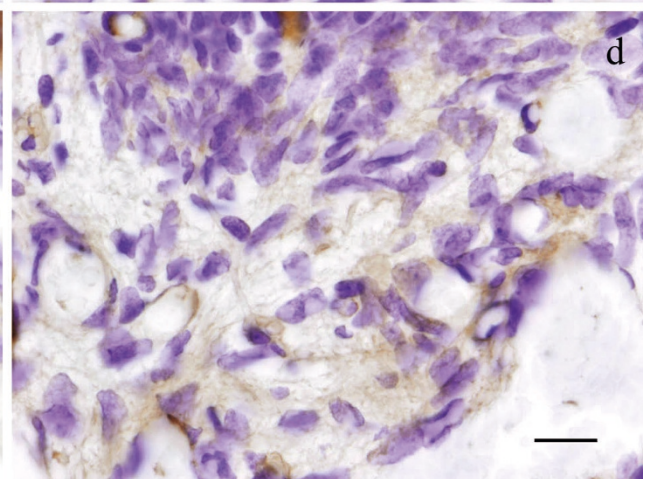
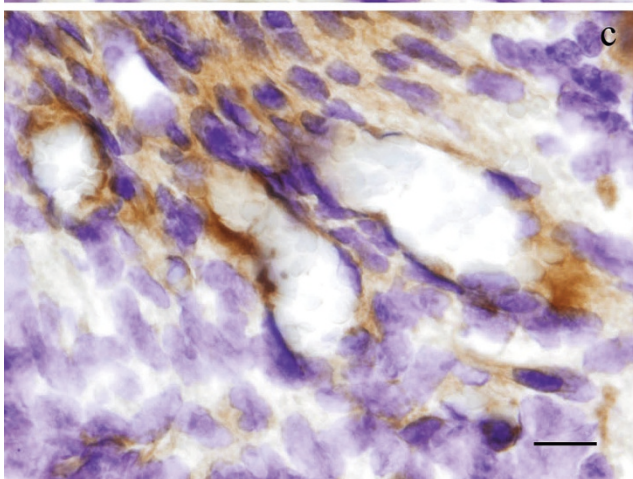
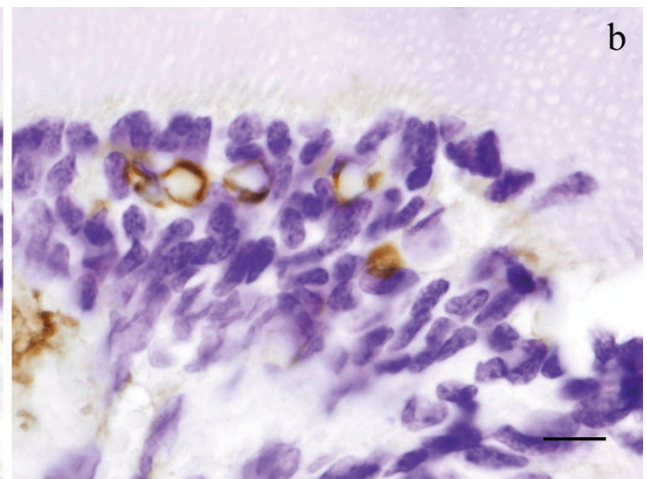
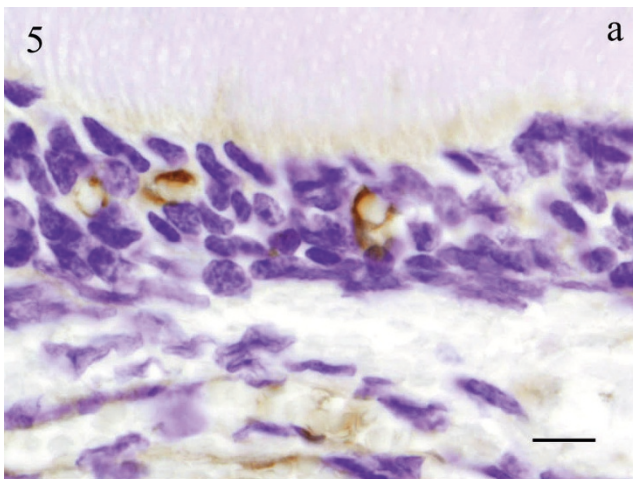
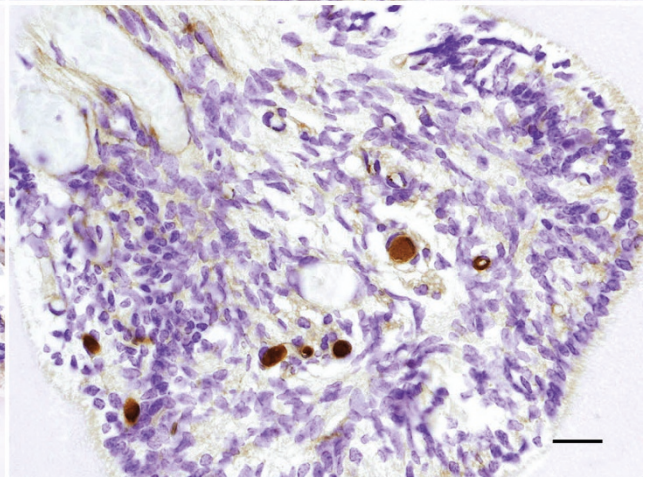
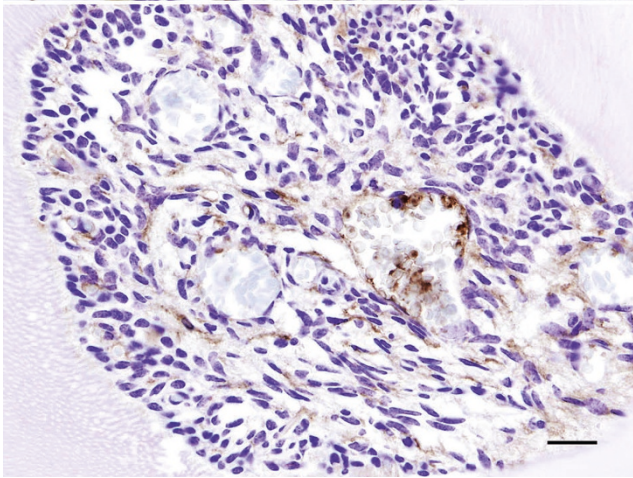
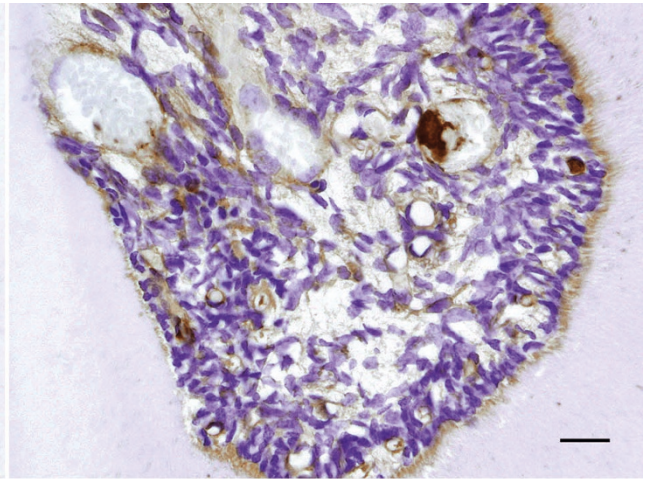
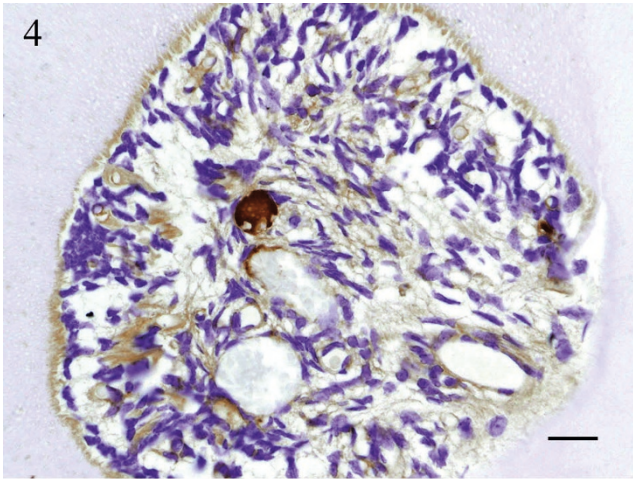


Fig. 3. Weakly positive staining present in some dental pulp cells (a: 30m-0m, Bar 20µm), pulp fibroblasts and capillaries (b: 30m-3h, Bar 20µm). Almost all pulp cells, including pulpal fibroblasts and endothelial cells show strong positive expression (c: 30m-24h, Bar 20µm) and weakly positive in some cells (d: 30m-1w, Bar 20µm).



which stimulated some endothelial cells and their reaction was to express HSP27. This may also be their way to recover from stress. However, the expression was reduced to the same level as that of the control group after a week suggesting that the injury or the stimulus was not that strong. For this, even if the 3 h group is compared to 6-fold stimulation over time, a similar trend was observed. Thus, separation of teeth creates a strong stimulus after about 1 week as indicated by the expression of HSP27 but the injury is not that destructive enough to the pulp.

### CONCLUSION

Immunohistochemistry was performed to examine the incidence of HSP27 expression. In the experimental groups, HSP27 expression was highest at 24 h after wedge removal and the expression remained the same or started to decrease thereafter. The expression decreased at the same level as that of the control group 1 week after wedge removal. Thus, HSP27 may serve as an indicator of stimulus strong enough to show its expression.

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